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FOUNDED BY JOHN D. ROCKEFELLER

THE METAMERISM OF NEPHELIS

With a Description of Nephelis Lateralis

(VERRILL)

A DISSERTATION

Submitted to the Faculties of the Graduate Schools of Arts, Literature, and Science, in candidacy for the degree of

DOCTOR OF PHILOSOPHY

(Department of Zoölogy)
December, 1896

BY

CHARLES LAWRENCE BRISTOL

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THE METAMERISM OF NEPHELIS.

A CONTRIBUTION TO THE MORPHOLOGY OF THE NERVOUS SYSTEM, WITH A DESCRIPTION OF NEPHELIS LATERALIS.

CHARLES L. BRISTOL.

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INTRODUCTION.

THE work which forms the basis of the present paper was begun in 1891 at Clark University, Worcester, and continued at the Marine Biological Laboratory at Woods Holl and Chicago University, and I wish to acknowledge here my indebtedness to the authorities of these institutions for the facilities and the Fellowship privileges granted to me. To Professor Whitman, at whose suggestion I began the investigation, I am deeply indebted for aid and encouragement and for many courtesies extended to me. I am under obligations to Dr. Wm. M. Wheeler

for aid and advice, and to Prof. S. A. Forbes, of the University of Illinois, for the privilege of examining the specimens of Nephelis collected by him under the auspices of the U. S. Fish Commission in the Yellowstone region in Wyoming.

HISTORICAL.

In 1767 Linné enumerated nine species of leeches in one genus, Hirudo. This classification was followed by most later authors, for example, Cuvier, Blumenbach, Carena, and Dumeril, until about 1817, when Savigny, in his *Système des Annelides*, announced the separation of Linné's genus into seven genera. The name Nephelis appears in this work for the first time, although Oken set this leech apart from Hirudo in 1815 under the name Helluo, which genus was to include all fresh-water leeches not provided with jaws. In 1818 Lamarck, at the suggestion of Blainville, proposed the name Erpobdella, which Blainville (1828) urged for acceptance because it contained the descriptive part "bdella." In 1826 Moquin-Tandon adopted Savigny's name Nephelis and continued it in the second edition of his *Monographie des Hirudinées* (1). The name has since become generally accepted, notwithstanding the fact that Oken's Helluo holds priority and Lamarck's Erpobdella is more descriptive.

The first description of Nephelis in America was made by Thomas Say (2) in 1824 under the name of *Hirudo lateralis*. In 1872 Verrill (3) changed this to *Nephelis lateralis*, which, for reasons given in another part of this paper, I have given to the leech I have studied.

METHODS.

The leeches are easily kept in aquaria, for which I used the low glass dishes known as crystallizing dishes, or white earthen-

[1] A. Moquin-Tandon: Monographie de la famille des hirudinées. Paris. 1846.

[2] T. Say: Major Long's Second Expedition to the Source of St. Peter's River, vol. ii, Appendix to the Natural History. 1824. (Republished in *Diesing's Système Helminthologique*, vol. i.)

[3] A. E. Verrill: "Synopsis of the North American Fresh-water Leeches." U. S. Fish Commissioner's Report for 1872–74. (Refers to the *American Journal of Science*, vol. iii, 1872.)

ware cooking dishes, known to the trade as nappies. In some instances I supplied the aquaria with a layer of mud and bottom *débris*, together with a few plants such as Ceratophyllum or Valisneria. When such an aquarium is covered with a glass plate it will keep fresh and clean for a long time and will furnish considerable food for the leeches. Generally, however, I used the plain dish, cleaning out the *débris* and slime and changing the water when necessary. I fed chopped fresh-water clams, but I do not doubt that salt-water clams will serve as well. I have kept individuals for over a year in normal condition and have raised many young under these conditions. When they are first transferred to the aquarium it must be covered for a day or two, to prevent escape. For superficial examination the leeches were killed in very dilute chromic acid, ⅙ to ⅓ per cent solutions. There is one period just before the acid penetrates very deeply when the surface markings stand out very clearly. The leeches usually extend themselves very well, and if killed in a wax tray they may be guided by pins. The best medium for histological details is a ¼ to ½ per cent solution of chromic acid, allowed to act for at least 24 hours. The stains used were borax carmine, Delafield's haematoxylin, and Bizzozero's picro-carmine. The macroscopic characters of the nerve chain were studied from maceration preparations. The leech is killed in a 20 per cent solution of nitric acid and left in it for from 24 to 36 hours, or until the skin and muscles can be easily removed with a porcupine bristle or a glass rod drawn out to a point. These were all carefully dissected away, leaving the chain entire. After thorough washing, the chain may be slightly stained in borax carmine and mounted in glycerine.

A number of details were worked out by the use of Haller's fluid. For example, the head was cut off, slit open on the ventral or dorsal side as wished, and killed in Haller's fluid while it was flattened under a piece of glass. After two days the specimen was transferred to glycerine.

The method that has given me the best results for nerves and sense organs is a gold chloride process kindly given me by Miss Julia B. Platt. It is so simple, so sure, and so exqui-

sitely delicate in some of its effects that it deserves extended use. It may be used with equal success on vertebrate or invertebrate, adult or larval tissue. It must be adapted to the tissue studied ; but this can easily be done after a few experiments. The formic acid appears to be the variable factor, and upon its strength and the time it acts depends the measure of success. I give the procedure applicable to Nephelis.

The leech is killed in a 10 or 15 per cent solution of formic acid, left from 5 to 10 minutes, and then put without washing into a 1 per cent solution of gold chloride for 25 minutes. From this it is transferred, without washing, into a large volume of 1 per cent formic acid, and left for 12 or 18 hours, or until reduction has taken place. It is next washed, passed through the alcohols to chloroform, and then imbedded in paraffin. The sections were cut 18 micra thick. The specimen will appear a rich purple when the reduction has taken place under the best conditions. The precautions are : to use small pieces of material, not thicker than 5 mm., to avoid maceration by reducing the strength of the formic acid and the time of action. My solutions were all well sunned, but no especial precautions were observed.

In tracing out the innervation of the somites it was necessary to examine long, continuous series of sections, and sometimes it was necessary to check results found in one somite by comparison with the next somite. The following method was used which would apply to other purposes. An ordinary library reference card, about 8 cm. by 10 cm., is ruled so as to include as many small rectangles in the same number of rows as the slide to be examined contains sections. The unused margin serves for making notes. An ordinary check mark denotes that the section occupying the same place on the slide that the rectangle does on the card has been examined but does not contain the element under examination. Initials, symbols, different colored pencils, etc., may be used to indicate various details, and each card is numbered the same as the slide. After a number of slides have been carefully plotted in this manner, the cards may be arranged in series and studied as a map. It furnishes an excellent reference-card system for any set of

serial sections, and permits a rapid glance at the order of sequence of any character in different somites or individuals.

Systematic.

Nephelis differs from nearly all other leeches in the external topography of the somite. While the somite in the Hirudinea, as a group, is characterized by prominent sense organs on the first ring, in Nephelis these are conspicuously absent, save on a few segments near the anus and in rare instances on a few rings near the mouth. The absence of these characters has compelled investigators to resort to other criteria for the determination of species, such as color markings, and the occurrence of four stripes of pigment on the dorsal side is sufficiently well marked to furnish a criterion of generic, if not of specific, value. In Europe the only well-established species is *N. octoculata* Bergmann. Blanchard (4) says: "Jusqu'a Savigny, la seule espèce admise sans contest était la *N. octoculata* Bergmann : Savigny a distingué plusieurs espèces basées exclusivement sur les differences de coloration : mais aucune de ces espèces nominale n'est representée et n'est surement reconnaisible. En outre de la *N. atomaria*, nous croyons pouvoir séparer de l'ancienne *N. octoculata* plusieurs autres formes spécifiques bien distinctes."

Moquin-Tandon in the first edition of his monograph accepts the description given by Carena for *N. atomaria* as a species, but in the second edition lowers it to the rank of a variety of *N. octoculata.*

The first mention of the genus in this country that I have found was made by Thomas Say (2) in 1824 under the name of *Hirudo lateralis*, and this was changed by Verrill (3) in 1872 to *N. lateralis.* Leidy (5) described a form (1870) under the name *N. marmorata.* Verrill describes four species of Nephelis found in the United States and says concerning three of them :

[4] R. Blanchard : "Courtes notices sur les Hirudinées, III. Description de la Nephelis atomaria Carena." *Bull. de la Soc. Zool. de France*, tome xvii, p. 165, 1892.

[5] Jos. Leidy : "Description of Nephelis punctata." *Proc. Acad. Nat. Sci. of Philadelphia*, p. 89, 1870.

"When a larger series of living specimens from various localities can be studied, the three preceding forms (*N. lateralis*, *N. quadristriata*, *N. marmorata*), admitted here as distinct, may prove to be mere varieties of one species, no less variable than *N. vulgaris* of Europe." The fourth species, *N. fervida*, is described from specimens taken from Lake Superior and has eight ocelli. I have not collected a Nephelis answering to this description.

The genus is widely distributed in the United States. My own collections have been made in Massachusetts, Connecticut, Illinois, New York, and South Dakota. I have received specimens from Mr. A. J. Hunter, of Toronto, collected near Toronto, Can., and Professor Forbes, of the University of Illinois, kindly loaned me for examination the specimens of Nephelis collected by him in the Yellowstone region in 1890. Verrill records collections from Maine, Massachusetts, Connecticut, New Jersey, Wisconsin, Nebraska, Colorado (at an elevation of 9000 feet on Longs Peak), and from the waters of Lakes Superior and Huron. The area included covers about 35 degrees of longitude and 10 degrees of latitude; it embraces the Atlantic slope, the Great Lake Region, the Missouri Valley, and the Rocky Mountains.

Investigations on my own collections lead me to agree with Savigny, Moquin-Tandon, and Verrill that it is difficult to distinguish species by the criteria used by them, color and color markings, and to disagree with the methods and results, published by Lindenfeld and Pietruszynski (6), who rely on these features exclusively. My first attempts to classify the specimens which I collected were naturally based on the descriptions given by previous investigators, but it proved so difficult a task to determine what value to place on the various statements of color, and so many of my specimens could with equal propriety be placed in either of two or three categories, that it became evident that some different method of diagnosis would be necessary. The necessity of going beyond color markings was plainly shown by the following experiments.

[6] Von Lindenfeld und Pietruszynski: "Beiträge zur Hirudineen fauna Polens." Reviewed by Nusbaum. *Biol. Centralblatt*, Bd. xii, p. 55, 1892.

I attempted to separate all the individuals collected from one locality near Worcester, Mass., according to color and color marks. I provided five aquaria and sorted each lot as I collected them until the whole number of individuals exceeded one hundred and twenty-five. It was very evident at a glance that the leeches in the first aquarium were light colored, and that those in the fifth were dark colored, but it was impossible to divide them so that each aquarium should be free from transitional forms. I repeated the effort on my collections from Wolf Lake, near Chicago, Ill., and with like results. The very light and very dark individuals were about equally rare, while the great bulk of each lot was made up of leeches varying in shade but having the same stripes more or less distinctly accented according to the amount of pigment present. These trials led me to adopt the method proposed by Whitman (7) and used by Blanchard (4). The method consists in determining the number of rings in the entire body and the limits of each somite. The first ring of each somite in the Hirudinea bears eight sense organs on the dorsal side, as Whitman has shown, four of which are serially homologous with the eyes.

The typical somite of Hirudo contains five rings. This number holds good throughout the middle body region, but falls to three towards the two ends, then to two, and finally to one. The amount and the manner of reduction vary in different genera, but are constant in any given genus. In Nephelis, also, the typical somite has five rings, but the limits of the somites and the number of rings in the terminal ones are not readily determined by the arrangement of the sensillae, for with certain exceptions mentioned hereafter these appear about equally prominent on every ring throughout the entire body. My first attempts to determine these points by means of the sensillae failed; I succeeded later in the following way.

When Nephelis is thrown into weak chromic acid, — 1/6 to 1/3 per cent solution, — there soon comes a time when the sensillae stand out with perfect distinctness; later the contrast in color between them and the surrounding surface becomes

[7] C. O. Whitman: "The External Morphology of the Leech." *Proc. Am. Acad. of Science*, vol. xx, p. 76, 1884.

less and less marked. At the time of greatest distinctness one may see that the sensillae are rather more strongly marked on the terminal somites, especially those at the hind end. It was here that I was able to find a starting point for determining the external metamerization. The 97th ring (Pl. VI, Fig. 3) was strongly marked with sensillae, and between the 96th and 97th rings were found the pores of the 17th and last pair of nephridia. These two conditions gave me a starting point from which I could fix with certainty the limits of the somites towards the anterior until the reduced somites of the eye region were reached. The nephridial pores were used as the limiting marks of the somite forward to the first pair of pores which lie between the 16th and 17th rings. From this point forward the sensillae aided somewhat, but the final results were based on the distribution of the nerves.

A careful reëxamination of my material now showed that, with one exception, I had collected or examined but one species of Nephelis. The exception was found among the leeches collected by Forbes in the Yellowstone region, and while the differences are such that I feel warranted in suggesting that proper study may show them to belong to another species, I could not, from the specimens at hand, determine this point.

The common species of Nephelis found east of the Rocky Mountains is the one that I have used in my investigations. The names adopted by Verrill (3) must, as he prophesies, be abandoned, and the name *Nephelis lateralis* be retained for this species so widely distributed over the United States.

Description.

The size of the sexually mature adult varies from 4 cm. to 10 cm. at rest. Anterior to the sexual openings the body tapers gradually to the mouth ; posterior to them the body continues about the same size until a little in front of the anus, where it narrows to the sucker. The transection of the body is lenticular, though in the pre-clitellar region it approximates a circle. The body flattens in swimming as it does in Macrobdella and Hirudo.

The color of the adults varies from a light chocolate brown free from any mark of pigmentation to almost a coal black free from any light areas. Between these extremes of very light and very dark all gradations of color and varieties of pigmentation may be found in individuals collected in the same pond or stream. The very light adults are comparatively rare, while among the young smaller individuals unpigmented specimens are quite common. The very dark adults are about as frequent as the very light adults, while a young dark individual is very rare. Most of the individuals that I have collected would fall into two sorts : those in which the pigmentation is diffuse, varying only in intensity through many shades, and those in which the pigmentation is arranged to a greater or less degree in longitudinal stripes. I have collected three individuals that showed definite pigmentation on the first ring of each somite, such as Blanchard (4) describes as constant for *N. octoculata.* Two were from Coonamasset pond near Woods Holl, and one was from Wolf Lake near Chicago. Other specimens with the diffuse type of pigmentation have shown a slightly accented color on the first rings of some of the somites, but not to the extent of defining all the somites. The ground color is either a light mahogany brown or a pale plumbeous gray. This may be observed on the ventral side, which is usually free from pigment. The color of an individual depends upon the amount of dark opaque pigment present either as small granular particles or as highly branching pigment cells. If the view of Graf (8) is correct, that the chloragogen cells wander into the epidermis and there break up, leaving their remains as pigment particles, then the wide variation in individuals taken from the same locality may be explained as individual variations in the manner of excretion.

It is interesting to note that the stripes of color so common in Nephelis lie in the lines of least resistance for wandering chloragogen cells. A reference to Pl. VIII, Fig. 18, shows five spaces between the bundles of long muscles on the dorsal side through which pass the dorso-ventral muscles, nerves, and

[8] Arnold Graf: "Beiträge zur Kenntniss der Exkretionsorgane von Nephelis vulgaris." *Jenaische Zeitschrift für Wissenschaft*, N. F., Bd. xxi, p. 163, 1893.

blood vessels, and it is directly over these or some of them that the pigment collects. I hope to make some further observations on this point by raising the progeny of one leech by themselves until they attain the adult markings.

Description of Nephelis Lateralis.

Since the analysis of Clepsine by Whitman (9) has given the prostomium the value of a somite consisting of one ring, I have followed the notation used by him and have counted the prostomium as ring No. 1 and somite No. 1.

Excepting the clitellum during its active phase, the body is not divided into obvious regions. The oral sucker is not prominent as in some species of Clepsine and the anal sucker is small, exceeding the body but little in width. The male orifice lies normally between rings 36 and 37. The female orifice lies normally between rings 38 and 39 (Pl. VI, Fig. 3).

The first pair of nephridiopores lies between rings 16 and 17 at the posterior edge of the 7th somite. There are four pairs of nephridia anterior to the male orifice, and these differ from the succeeding nephridia by reduction of certain parts. The pores of the first pair of nephridia behind the male pore lie between rings 36 and 37, about midway between the median plane and the margin, and these are followed in regular order, at intervals of five rings, by the remaining pores. The last pores lie between rings 96 and 97, and the whole number of pairs of nephridia is seventeen. The anus is dorsal and lies behind the 104th ring.

The clitellum consists of fifteen rings — from 28 to 42 inclusive. It includes the last four rings of somite X and the first of somite XIII. It is plainly visible only during sexual activity; at other times it can scarcely be distinguished from the adjacent rings. In the active condition it is paler in color and may be swollen so as to become larger than any other part of the body.

[9] "The Metamerism of Clepsine." *Festschrift für Leuckart*, p. 395, 1895.

Somites.

The number of rings in the typical somite is five, but this number is reduced at each extremity. Unlike Clepsine, Macrobdella, Hirudo, and some other leeches, Nephelis does not have the first ring of each somite, except in the anal region, marked by especially large sensillae, and the study of a large number of individuals showed the arrangement of sensillae to be constant in this region. The last nephridiopores lie between rings 96 and 97, and 97 is well marked by sensillae (see Pl. VI, Fig. 7). This, then, is the first ring of a somite. The next four rings following have no prominent sensillae, but ring 102 is again strongly marked with them; 103 is a broad double ring; 104 is another double ring, the latter half of which bears sensillae. The anus sometimes divides this part of the ring and so comes to be bounded anteriorly by 103, but generally a thin portion of 104 forms the anterior lip of the anus. Rings 102, 103, and the anterior half of 104 make up a pre-anal abbreviated somite, while rings 97 to 101 form a complete post-nephridial somite. Now going forward as far as the first pair of nephridiopores (Fig. 3) the somites may be readily traced by the nephridial openings, and they consist of five rings each. At this point another criterion enables us to determine one complete pre-nephridial somite. The ganglion in each typical somite lies almost wholly in the first ring. If we count five rings forward from the first nephridiopores, we find the first ganglion of the nerve cord lying in this ring, the 12th. The innervation of these five rings also proves that they make up a complete somite. To recapitulate: The somite anterior to the anus is reduced to two and a half, morphologically four, rings; thence forward to the 12th ring inclusive we find eighteen complete somites, innervated by the eighteen separate ganglia of the nerve cord.

The reduced somites of the head region are innervated from the "brain" and sub-oesophageal ganglia, while the reduced somites of the anal region are innervated from the anal ganglia.

Head Region.

The innervation of the rings of the head region shows, as will be demonstrated later, the limits of the reduced somites to be as follows : (Fig. 3) I consists of the prostomium ; II of rings No. 2 and 3 ; III of a single broad ring, No. 4 ; IV of a single ring, No. 5 (this ring lies in the plane of flexion of the body on the oral sucker and is very narrow) ; V consists of three rings, Nos. 6, 7, and 8 ; VI consists also of three rings, Nos. 9, 10, and 11.

Anal Region.

In the anal region (Pl. VI, Figs. 3 and 7) the innervation shows the limits as follows : XXV consists of rings 102, 103, and the anterior half of 104. XXVI consists of the posterior half of 104 and 105. 105 is a broad ring which in some individuals shows a tendency to divide into two, sometimes three, rings. It lies in the plane of flexion between the body and the anal sucker. XXVII consists of 106, the last ring of the body and the dorsal area of the sucker. XXVIII to XXXIV consist of the sucker disc. External evidence of this is found in the six radial lines of sensillae on either side of the median plane.

Summary.

The number of rings is 106 from prostomium to sucker. The first pair of eyes (Fig. 4) lies in the 2d ring ; the second pair lies wholly in the 4th, while the third pair lies usually between the 4th and 5th rings. The clitellum consists of fifteen rings, Nos. 28 to 42 inclusive, or the 2d to 5th ring of somite X, the ten rings of XI and XII, and the 1st ring of somite XIII. The male orifice lies, usually, between the 36th and 37th rings. The female orifice between the 38th and 39th rings.

The anus opens in the hinder portion of the 104th ring, or between the 104th and 105th rings.

The first nephridiopore lies between the 16th and 17th rings ; the last and 17th nephridiopore lies between the 96th and 97th rings.

The head region consists of the first six somites, comprising the first eleven rings. The first body ganglion lies in the 12th ring, the 1st ring of somite VII.

The 18th and last body ganglion lies in the 97th ring, the 1st ring of somite XXIV.

The body region extends from ring 12 to ring 101, somites VII to XXIV inclusive.

The anal region extends from ring 102 to the disc of the sucker; somites XXV, XXVI, and XXVII.

The sucker contains seven somites, XXVIII to XXXIV.

Habitat.

Like other leeches, Nephelis keeps its body for the most part in the dark, and must be sought for according to the conditions of the bottom of the pond or stream. In a stony brook or pond beach they may be found adhering to the underside of the stones; on a sand beach unshaded from the sun they bury themselves almost completely in the sand, projecting their heads at short intervals in search of food. Where the overhanging trees have dropped their leaves into the water they will be found on the underside of the leaves. They may be found on the underside of the water-lily leaves, on floating pieces of wood, and between the bark and the wood of rotting, water-logged branches of trees.

They thrive under widely different conditions of water, soil, and temperature, so long as food is obtainable. I have collected them in the Charles River at Cambridge, Mass., during low water in midsummer, when the river was reeking with sewage and the chemical wastes from paper mills, while the temperature was but a few degrees lower than that of the air. Yet, within a stone's throw of the river bank I have collected them quite as readily in a clear spring-water brook, in which the water was so cold that collecting in it was almost painful. The abundant food supply appeared to be the only feature common to the two places.

The character of the bottom of a pond seems to be an indifferent factor, for in the same pond they may be as numer-

ous on a mud bottom as on a sand bottom. This was a matter of surprise to me until I found the explanation. I noticed that I invariably made the best collections on the shore that looks towards the prevailing summer winds ; that is, the shore towards which the surface current flows, bringing with it crustaceans, dead fish, and various other food materials. The windward shore is almost always barren of Nephelis, for the water on that shore is the cool water of the deeper parts and is poor in food for Nephelis. That this food supplying current is the important factor in influencing the distribution of Nephelis is beautifully demonstrated in the small fresh-water ponds near Woods Holl.

These ponds lie in basins scooped out by glacial action, and many of them have no outlet. Some are nearly circular, others are elliptical or long and narrow. The surrounding hills are comparatively high, and the direction of the prevailing wind over the pond is frequently determined by the trend of the lower land or valley near the pond. This exposure to wind varies in different ponds lying near together, and Nephelis are always more abundant on the lee shore. In brooks they are usually more abundant near the mouth of the stream, whether it flows into another stream or into a pond. This is explained in the same way. Food brought down by the brook is more plentiful at that point than at any other.

Habits, Food, etc.

Nephelis, like Aulostoma, is non-parasitic and differs from the parasitic leeches in many of its habits. It does not readily leave the water like Hirudo or Macrobdella, and in confinement it seldom attempts to leave the aquarium after the first twenty-four hours, if there be plenty of food. It swims freely and rapidly with the same undulating movement that Hirudo employs. In creeping it never brings the anal sucker up to the oral sucker as Clepsine, Hirudo, and Macrobdella, but usually attaches it about halfway between the two in the outstretched body. In common with other leeches, Nephelis has the habit of fixing itself by the anal sucker and then undulating

its body as in swimming. In repose it commonly seeks shelter under a stone, a leaf, a clump of weeds, or in the upper layer of mud or sand at the bottom, exposing only the anterior third of the body. It rests in this position for comparatively long periods and seems, at times, to be sleeping, or at least so sluggish as to require considerable stimulating before it responds. Sometimes it rests curled up in a spiral with its head in the center and attached by its anal sucker. When undisturbed and active it creeps in search of food and stopping now and then it attaches itself by the anal sucker and explores an arc of the circle of which its body is the radius. The head sways from right to left, up and down, while the body is extended gradually to full length ; then the body is shortened and moved through a small angle and the first process is repeated.

When hungry, either at rest or creeping in search of food, Nephelis is quick to perceive its presence ; but while swimming it seems to be less attracted, although it may swim nearly in contact with the foodstuff. My experiments were made on leeches in aquaria, purposely left without food for some days. Leeches fresh from the pond gave practically the same results as leeches that had been kept in confinement for long periods. When the water is about 3 cm. deep an individual at rest on the bottom will perceive a portion of food let down gently overhead almost as soon as it touches the surface ; after a short interval, fifteen or twenty seconds, the leeches lying from 4 to 6 cm. away will give evidence of perception and they will set out to find it. If at another time, when the leeches are bunched together in a mass, the food be placed about 10 cm. away, a minute or a minute and a half may elapse before one shows any sign of awakening and starting in search of the food. Others follow more or less rapidly at intervals. Under these conditions there seems to be some evidence of a sense of direction, but it is vague, if not mere chance. Some, not always the first ones, will start off in the proper direction; others will stray afar ; some will come within 1 cm. of the food and pass on without noticing it ; others will start to swim briskly in irregular paths as if to trace the scent, and when near the food will suddenly settle down, fix the anal sucker,

and explore. They are as likely to explore away from the food as towards it. If, while swimming, any portion of the body touches the food, a leech will often perceive it, stop short, and feed on it.

If a leech is feeding, any other leech that comes in contact with it perceives instantly what the other is doing and rapidly creeps along its body to partake of the meal. I have frequently started up every individual in a bunch of a dozen or more, by gently pushing a morsel to one which projected its head a little beyond the others. The first motion of seizure would be enough to set the whole bunch in commotion. If a bit of food be gently placed on the back of an individual at rest, it will often whirl rapidly about and seize it, though it remains indifferent to another leech creeping over the same place. If, in a clear aquarium containing some hungry Nephelis, the finger be rubbed over the bottom and continued up the side out of the water, the leeches as they creep along the bottom will perceive the scent and follow the trail, even to some distance out of water.

These experiments indicate the same general conditions of perception as Professor Whitman has found in Macrobdella (10).

In the summer time Nephelis lives in the shallow waters of the pond, but in winter it goes down to the deeper parts or into the mud of the edges if there is a good food supply. I have found them in midwinter in seven feet of water when the ice was 25 cm. thick, and in another place in the mud near the edge when the ice was 50 cm. thick, leaving only 8 or 10 cm. of water over the mud. In both cases the individuals were as active as in summer, and some of them laid eggs after being a few weeks in the aquaria. These developed and produced normal individuals which in one instance gave me a supply of small individuals very opportunely.

Nervous System.

When this work on the nervous system of Nephelis was begun, the chief object in view was to determine the innervation of the somites as a means of elucidating the metamerism of Nephelis.

[10] "The Leeches of Japan." *Quar. Journ. Micr. Soc.*, vol. xxvi, p. 317, 1886.

Professor Whitman was making a study of the nervous system of Clepsine (9) for this purpose, and suggested that the same be done with Nephelis, in order to bring the two genera into comparison. In order that the relations between the two may be clear, I present the following summary of his paper, so far as it bears on this question.

He presents some considerations drawn from embryological evidence to show that the head includes a number of true metameres. "Does it include anything more?"

"In the adult head we find the segments fairly well defined behind the eyes, but how far the metameric division extends into the prae-ocular region remains to be determined. With reference to the origin of the head, we are compelled to take one of two views. The head consists either (1) of a non-metameric lobe plus a number of metameres originally belonging to the trunk, or (2) of such metameres only, the non-metameric head element of the ancestral form having been lost or incorporated in the first metamere."

Each body neuromere in Clepsine, disregarding the longitudinal nerve cords which fuse regularly at the level of each metameric center, comprises three pairs of nerves and six ganglionic masses, each mass being contained in its own capsule. Two of these are always ventral and median, the remaining four are arranged in pairs, two on either side above the nerve roots. The sub-oesophageal ganglia readily show their metameric origin; the ventral capsules of the body neuromeres persist, arranged in a median row with only the two anterior capsules crowded into bilateral positions. The corresponding lateral capsules are readily identified in the 6th, 5th, and 4th segments, while the others in the 3d and 2d have been crowded out of the places they would naturally occupy. The nerves from this region are also identified as containing the elements of the single neuromere. VI, V, and IV have three roots each; III shows only two roots, and II issues as a single root, which soon divides into two branches. Sections show very plainly the presence of five nerve roots, each with its pair of median nerve cells. Thus the evidence is conclusive that the sub-oesophageal region consists of five metameres (II to VI).

"The surprising thing is that we have left what seems to be the exact equivalent of a trunk neuromere; *one pair of nerves* (1) *and six ganglionic sacs,* of which two are median and four are lateral. Whether there is a pair of 'median nerve cells' connected with this part of the nervous system, I cannot say. I have not found them, but my search has not been exhaustive. The equivalence in other respects is so complete that there seems to be no escape from the conclusion that *the ganglionic centers of the ventral cord are simple repetitions, element for element, of the 'brain.'* The nervous system is made up of segments of equal morphological value throughout. It must be regarded then either as a series of 'brains' or as a series of ventral neuromeres, one or more of which have been carried secondarily to the dorsal side, and which here take the place of a brain that has been lost or confounded with the metameric system. That a portion of neuromere II has suffered transportation from the ventral to the dorsal side is certain; but the development of the supra-oesophageal system does not permit us to believe that neuromere I was ever post-oral in position. Allowing that it represents genetically the annelid brain, as it certainly seems to do, the ventral cord must be regarded as a chain of brains. The *dorsal* position of the brain signifies nothing more than that the anterior end of the double nerve cord has been bent upward from its prae-oral and ventral position and slipped backward over the oesophagus."

In the caudal region, although the concentration is quite as great as in the head region, the elements of the neuromeres are plainly resolvable. Each neuromere is complete in the number of capsules and the nerve roots, which, however, are here reduced to two. The whole nerve chain is divisible into three portions: the head with six neuromeres, the trunk with twenty-one, and the caudal disc with seven, making a total of thirty-four neuromeres. Referring to Pl. I (Pl. IV here), the innervation of a typical body somite is made clear. We find the nerve divided into *three* distinct parts which we may designate as anterior, middle, and posterior nerve, respectively. "A glance will make clear one very interesting feature in the distribution of these nerves. They *innervate three successive rings,*

the first and second of their own segment, and the third of the preceding segment. The distribution is thus triannulate and dimeric."

Passing to the head region, we find a number of interesting modifications of the plan found in the body somites. Nerve VI has three parts, "but they are no longer the precise equivalents of 'anterior,' 'middle,' and 'posterior' nerves. What before appeared as the dorsal branch of the posterior nerve now appears as the middle nerve, supplying the same sense organs as before and, in addition, the inner lateral sense organ of segment V. The third nerve has no dorsal branch except the short one to the outer lateral sense organ. It has two main branches, however, one of which takes the place of the 'middle' nerve, the other that of the 'posterior' nerve. The first nerve alone remains the unchanged 'anterior' nerve. The branch running to the inner lateral sense organ (*i.l.*) of segment V belongs, according to what we saw in typical segments, not to segment VI, but to segment V.

"In segment V we find three nerves, but their composition and distribution depart still further from the typical arrangement. This nerve, as shown in Pl. I [Pl. IV here], gives off a number of motor branches, and then passes to the outer lateral and marginal sense organs and the labial organs of four rings (8–12). It innervates then the first and second rings of its own segment, and two rings (9–10) of segment IV. It corresponds then to the 'middle' nerve in the trunk region, but contains also fibers belonging to three other nerves, namely, the 'posterior' nerve of the preceding segment, and the 'anterior' and 'posterior' of its own segment. Just above and a little in advance of this root appears another quite strong nerve, which rises and passes forward over the lateral angle of the supra-oesophageal ganglia. This nerve divides just in front of the head ganglia, sending one branch to the inner lateral organ of segment IV, and the other to the median organs of segments IV and V. This nerve then corresponds to the dorsal sensory branch of a 'posterior' nerve, and includes so far as it goes the fibers of two such branches, for segments IV and V.

"In segment IV we find only two nerves, one small motor, corresponding to the 'anterior' nerve, and one large nerve

which, after giving off several motor nerves, runs to the labial sense organs of three rings (6–8) and to the outer lateral organ of ring 8 in its own segment. This nerve corresponds in the main to a 'middle' nerve. The sensory 'dorsal branch' of the 'posterior' nerve of this segment, as we have seen, is united with the corresponding nerve of segment V.

"In segment III we find only two nerves, corresponding with the two seen in segment IV. Where is the sensory 'dorsal branch'? On examining nerve II, we find it contains the missing nerve united with the corresponding nerve of segment II. Nerve II supplies not only the rudimentary eyes (median sense organs) of its own segment, but also the pair of large eyes and the inner lateral organ of segment III. One of its two main branches supplies the outer lateral organ and the labial organs of segment II.

"Nerve I innervates the median, the inner lateral, and labial sense organs of the most anterior division of the head."

This species shows also very plainly that some of the metameric sense organs acquire eye-like properties in the head region which gradually increase towards the anterior somites. "In no other species hitherto described do we find the sensillae passing by such gradations into the eyes. *The serial homology of these organs with the eyes is then a fact demonstrated not only by the embryonic development, but also by the structural gradations in the adult animal.*"

In the concluding portion Professor Whitman reviews the evidence derived from the innervation of the head region and says: "The morphological equivalence of segment I with the following segments is evident to a degree that is really astonishing. It makes no departure from the typical trunk segment which is not led up to through gradations represented in the segments immediately following it."

THE NERVOUS SYSTEM OF NEPHELIS.

The nervous system of Nephelis may for convenience be divided into two parts: that portion which responds to external stimuli and coördinates the muscles of locomotion, the central

nervous system; and that portion intimately connected with the control of the organs of internal life which I shall call the sympathetic system. These two parts differ widely in certain characteristics of structure as well as of function. The central nervous system is strongly metameric throughout its length. Its cells are relatively larger and are referable to the unipolar and bipolar types for the most part. The fibers of these cells always tend to run in bundles and never to form plexuses. The sympathetic, on the other hand, is free from any discoverable trace of metamerism; its cells are small and frequently multipolar, and the fibers always tend to form plexuses (Pl. VIII, Fig. 19).

The Central Nervous System.

The entire ganglionic chain in Nephelis, as in other leeches, is contained in the ventral blood sinus, which, according to Bourne and others, is one of the vestiges of the original coelomic cavity. This sinus runs directly under the alimentary canal and is readily distinguished by its dark pigmentation and the swellings within which lie the ganglia.

The anterior end of the chain, called the sub-oesophageal ganglia and the brain, consists of a mass of neuromeres more or less completely fused together, and forming a collar about the oesophagus. The posterior end, called the "anal ganglia," consists likewise of a number of neuromeres more or less completely fused. Between these terminal portions lie eighteen neuromeres joined each to the next by two connectives. Between these, and dorsal to the axis of the chain, lies a small bundle of fibers known as the median nerve, or Faivre's nerve. These connectives are longest in the mid-body region and decrease in length towards either end of the body, becoming almost nil in the most fused parts at both extremities. Within each connective lies a "colossal axial" cell, the nucleus lying about midway between the neuromeres, as has been described for other leeches.

At the points of junction between the connectives and the neuromeres the fibers of the connectives do not separate into small bundles as they do in Hirudo and Macrobdella, but each continues into the body of the neuromere as a single bundle.

A Typical Neuromere.

In order to analyze the "brain" and the "anal ganglia" it is necessary to know the component parts of a typical neuromere and to grasp their relations to each other under normal conditions.

The general shape of a ganglion is that of a flattened ellipsoid, the long axis of which is parallel to that of the body; the ventral surface being slightly more convex than the dorsal (Pl. VI, Fig. 9). Each ganglion gives rise to two nerves on each side which leave the ganglion and proceed for a short distance in a horizontal plane, and then branching, go to the dorsal or ventral side.

The anterior nerve, however, is not a single nerve. It results from the fusion of a ventral and a dorsal root, the fusion taking place almost immediately after their departure from the body of the ganglion (Pl. VI, Fig. 9). This fact enables us to homologize the two lateral nerves of Nephelis with the three of Clepsine as follows: I and II in Clepsine are represented by I in Nephelis. III in Clepsine is II in Nephelis. This homology is also shown by the correspondence of the areas innervated by I in Nephelis and I and II in Clepsine (Pll. IV and V). I have not been able to find evidence of the similar origin of the anterior nerve in Hirudo or Macrobdella, and this fact suggests that Nephelis is an intermediate form between the Clepsinidae and the five-ring leeches.

Between each pair of the lateral nerves, and near the ganglion, lies a bipolar cell, the principal prolongations of which pass outward along the trunks of the lateral nerves for a short distance and then fuse with them so as to be indistinguishable from them. This cell is found in other Hirudinea and has been called from its discoverer "Leydig's cell." Its presence throughout the entire ganglion chain, its variation under different conditions, and its possible relations to other extraganglionic cells are of sufficient interest to demand for it separate consideration.

The nerve cells of the ganglion, with the exception of "Leydig's cell," are gathered into six groups or clusters lying

outside of the central fibrous portion in capsules as in the other Hirudinea. Pl. VI, Fig 9, shows the general arrangement. Two clusters lie on the ventral surface in the median line (one anterior and the other posterior) and two clusters on each lateral face. The anterior lateral clusters are anterior to the anterior nerves, and the posterior lateral clusters lie between the two nerves. These lateral clusters rise slightly above the dorsal surface of the body of the ganglion, and their posterior edges are notched by the lateral nerves as they pass out from the ganglion (Pl. VI, Fig. 12). The number six is constant in the whole chain and the position is also constant except in the supra-oesophageal ganglia or "brain." In the "anal ganglia" the lateral clusters tend to become dorsal towards the posterior portion owing to compression, but they are perfectly recognizable and referable to their proper neuromeres (Pl. VI, Figs. 14 and 15).

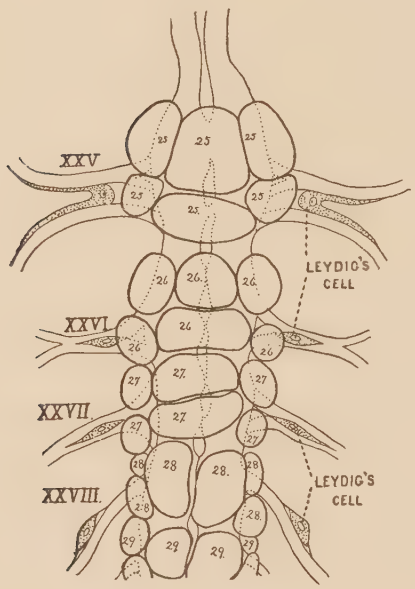
Leydig's Cell.

Lying between the two nerve trunks of either side of the ganglion, and nearly in contact with the posterior lateral capsule, lies a large bipolar cell whose prolongations follow along the lateral nerves as they pass outward, and finally fuse with them (Pl. VI, Fig. 9). This cell was described first by Leydig in *Hirudo medicinalis;* it was called "Leydig's cell" by Hermann (11), and is found in all the Gnathobdellidae in the same relation to the ganglion. I have not been able to find any trace of fibers going to the ganglion; so far as I have traced them they all pass outward along the nerves. This cell, the significance of which is as yet entirely unknown, is constant throughout the central nervous system. I have found it in every neuromere of the body. In the first four neuromeres it lies upon the fused nerves at some distance anterior to the "brain" (Pl. V, Fig. 2). In the fifth neuromere (Pl. VI, Fig. 11) the cell is found at the angle formed by the separation of the hitherto fused portions of the nerve trunks. In the sixth neuromere, the last nerve of the sub-oesophageal mass, the cell

[11] Ernst Hermann: Das Central Nervensystem von Hirudo medicinalis. München. 1875.

lies much closer to the mass and exhibits more of the characters of the normal cells found in the body ganglia.

In the "anal ganglia" (Pl. VI, Figs. 14 and 15, and Fig. 1 in the text), the first neuromere, XXV, the cell is normal. In the second, XXVI, the two nerves partly fuse at a little distance from the margin, and continue thus for a short distance, when they become fully separate. Within this region of partial fusion "Leydig's cell" is found lying between the two trunks compressed and changed into a spindle-shaped cell; the prolongations extending median and lateral as in the cells of the first four neuromeres. In the third "anal" neuromere, XXVII, the cell appears in the same relative position, but is more compressed and elongated. Good histological preparations of these cells show that the size and structure of the nucleus and the nucleoli are identical with those of a cell from a mid-body region.



Text-fig. 1. — The four anterior neuromeres of the "anal ganglia" seen from the ventral side, showing the stages of compression of "Leydig's cell" till it appears outside of the fused trunk in XXVIII (1st anal in Clepsine) and the succeeding nerves. (From a camera drawing of a nitric acid preparation. The details of the "Leydig's cell" were supplied from sections.)

The fourth, XXVIII, and succeeding neuromeres, XXIX to XXXIV, innervate the sucker. The fusion here is as complete as in the first four neuromeres, and the "Leydig's cell" has been pushed out, in the more complete fusion of the nerve trunks, until it lies completely outside of and upon the nerve, about midway from the anal ganglion to the edge of the sucker; the prolongations extending, as before, median and lateral. In these most posterior neuromeres the size and

structure of the characteristic features of the cell remain unchanged from those of the normal mid-body cell.

Median Nerve Cells.

Within the fibrous body portion of the normal ganglion, near the median plane, lie two "median nerve cells," one slightly anterior, the other posterior, to the center. They are found in all the Hirudinea and have been described by several authors. Retzius (12) and Biedermann (13) show them in their figures of Hirudo obtained by methylen blue, and they continue to appear forward in the sub-oesophageal ganglia. In my analysis of the "brain" I shall speak of these in detail. I have also found them in the anterior neuromeres of the "anal ganglia," but I am not able to say whether they are present in the posterior neuromeres or not.

The Fibrous Portion.

The fibrous part of the ganglion occupies the axial portion and, macroscopically, appears to consist of thickenings of the two connectives that afterwards fuse. It is perforated by two small holes which lie close together on either side of the median plane at the level of the anterior nerves. These perforations persist in the fused portions of the nerve chain and afford good evidence of the fusion of originally separate neuromeres.

According to Biedermann (*l. c.*) and Retzius (*l. c.*), this fibrous part is made up of fibers from three different sources : (1) from the connectives, part of which continue through the ganglion ; (2) the efferent fibers from the neurones, which fill the six capsules of the ganglion ; and (3) the afferent fibers, which are the central termini of neurones whose trophic centers lie outside of the ganglia.

The first two sources are readily demonstrated, but Retzius failed to find the source of all the fibers in the third set. His

[12] G. Retzius : Biologische Untersuchungen. Neue Folge, 2. Stockholm. 1890.

[13] W. Biedermann : "Ueber den Ursprung und die Endigungsweise der Nerven in den Ganglien wirbelloser Thiere." *Jenaische Zeitschrift für Naturwiss.*, Bd. xxv, 1891.

figures are very clear and show the structure of the fibrous portion of the ganglion with great detail.

He traces out the course of the axis cylinders from the cells of each capsule, and of those fibers whose trophic centers lie outside of the ganglion. He separates these fibers into six groups, five of which come into the ganglion by way of the connectives and one by way of the lateral nerves. This last group, the sixth of Retzius, is of peculiar interest, because while Retzius was drawn by his examination farther and farther from the ganglion to search for the cell-bodies of this class of fibers, until he reached the epidermis, he did not succeed in finding them.

He says (N. F., ii, p. 21): "Was stellen nun diese Fasern dar? Sie sind offenbar Nervenfasern, welche peripherisch verlaufen. Wo sind aber ihre Ganglienzellen? Da ich bei den Crustaceen ähnliche, durch die peripheren Nervenzweige aus den Ganglien des Bauchstrangs, austretende Nervenfasern mit grossen in den Ganglien befindlichen Ganglienzellen in Verbindung gefunden hatte, so schien es mir auch bei den Hirudineen möglich zu sein, dass die fraglichen Fasern von intraganglionären Zellen entspringen könnten. Es erwies aber durch zahlreiche Versuche, dass dieses nicht der Fall war; keine Ganglienzellen konnten mit ihnen in Verbindung angetroffen werden.

"Die fraglichen Fasern treten offenbar von der Peripherie her in die Ganglien hinein, um hinter der geschilderten Verästelung sich in ihre Punktsubstanz aufzulösen.

"Der von Hermann u. a. gemachte Befund grosser Ganglienzellen im Verlauf der peripheren Nervenzweige erklärt aber in sehr plausibler Weise ihre morphologische Bedeutung; sie müssen eine Art von Nebenfortsätzen dieser peripheren Ganglienzellen darstellen, welche durch sie die contactartige Verbindung mit den Elementen der Ganglien, d. h. dem centralen Nervensystem, aufrecht erhalten. Ich versuchte nun, durch Methylenblaulösung die fraglichen Ganglienzellen und ihre Fortzätze zu färben, aber der Pigmentreichthum und die Scheidenbildungen verhinderten leider bei Aulastoma und Hirudo die endgültige Lösung dieses interessanten Problems."

I believe that I have found the source of these fibers in the bipolar cells that lie in the intermuscular nerve ring, the description of which will be given later.

Innervation of a Body Metamere.

As I have said before, the ganglion lies in the first ring of the somite and the two lateral nerves pass out, for a little distance, in a horizontal plane and at right angles to the long axis of the body. Then they divide into dorsal and ventral branches, and again divide and subdivide to innervate the various organs, as described in detail below. The first and most striking fact is that the distribution is morphologically identical with that of Clepsine, and the second that it confirms Professor Whitman's explanation of the derivation of the five-ring metamere from a three-ring type: *e.g.*, Clepsine.

A glance at Pl. V, metamere VIII, will show that the anterior nerve innervates the 4th and 5th annuli of the preceding metamere on the ventral side and the extreme lateral sensillae of the 1st annulus of its own metamere, and sends fibers to the intermuscular nerve ring in the 5th annulus. The posterior nerve sends one ventral branch to the sense organs on the ventral side of the 3d annulus and two ventral branches to the intermuscular nerve ring in the 2d annulus, one of which by subdivision makes two connections with the nerve ring. The principal branch of the posterior nerve is dorsal, and this branch innervates, first, the few dorsal sensillae on the 4th annulus of the preceding metamere; second, the dorsal side of the nerve ring in the 5th annulus; third, the large sensillae in the 1st ring of its somite; fourth, the dorsal side of the nerve ring in the 2d annulus; and fifth, a few dorsal sensillae in the 3d annulus. A comparison now with Professor Whitman's work on Clepsine will show how completely identical the distribution is (Pll. IV and V). The 4th and 5th annuli are morphologically the 3d annulus of Clepsine; they are innervated by ventral portions of the anterior nerve, as in Clepsine. The branch of the anterior nerve in Nephelis that represents the middle nerve in Clepsine innervates exactly

the corresponding area in Nephelis, the ventral side of the 1st annulus together with the outer lateral sensillae. The posterior nerve in Nephelis, as in Clepsine, is the principal sensory nerve, innervates dorsal sensillae in all five rings, and ventral organs in annuli 2 and 3 (Clepsine 2).

Remembering, then, that annuli 4 and 5 represent annulus 3 in Clepsine, that 2 and 3 represent annulus 2 in Clepsine, together with the homology of the nerves, the anterior nerve of Nephelis representing the anterior and middle nerves of Clepsine, we may use Professor Whitman's words (9, p. 388) to describe the distribution of the nerves of Clepsine for Nephelis as well: "They innervate three successive rings, the 1st and 2d of their own segment and the 3d of the preceding segment. The distribution is thus triannulate and dimeric."

The Innervation of the Terminal Somites.

We are now prepared to understand the modifications of the plan of innervation found in a body somite as found in the somites innervated by the more or less completely fused terminal neuromeres in the head region, somites I to V, and those in the anal region, somites XXV to XXXIV. These I shall call terminal somites for convenience. Of these two groups, those of the anal region present less departure from the normal and hence will be described first.

The Anal Region.

As I have stated briefly elsewhere, the posterior portion of the nerve chain is sometimes called the anal ganglia. As Whitman and others have shown in other leeches, so in Nephelis the nerve chain in this region consists of neuromeres more or less fused together but retaining their fundamental characteristics to such an extent that they can be easily identified. Pl. VI, Figs. 14 and 15, show the dorsal and ventral views. The number of neuromeres is ten, — the three anterior being less modified by the fusion than the remaining seven. Koehler (14)

[14] R. Koehler: Recherches sur la structure du système nerveux de la Nephelis. 8°. Nancy. 1882.

is quoted by François (15) as assigning nine neuromeres to the anal ganglia. The 1st anal neuromere, XXV, innervates the first of the posterior terminal somites (Pl. V, Fig. 7), consisting of annulus 102, the somewhat double annulus 103, and the anterior half of 104. The 2d anal, XXVI, innervates the anus-bearing somite, the posterior half of annulus 104, and the broad annulus 105, which often shows traces of doubling. The 3d anal, XXVII, innervates the last somite preceding the sucker, which consists of annulus 106 and the "acetabulum," the area lying between the last annulus and the sucker. This area lies in the plane of flexion of the body on the sucker and has lost all trace of annulation. The succeeding seven neuromeres innervate the sucker. The 4th anal, XXVIII, innervates the anterior part of the sucker, immediately on either side of the median plane. The last anal, XXXIV, innervates the posterior part of the sucker in the same way, while the intermediate neuromeres supply the rest of the sucker radially between these parts. The XXVth or 1st anal somite resembles in every particular a normal body somite. The connectives to the XXVIth are very short and broad, and they become shorter and more nearly uniform in breadth with the body of the ganglion as we continue backward. They preserve their characters as connectives, however, as is shown by the oblong slits in the central fibrous portion, until the XXXIId neuromere is reached, when the slits cease. The nerves of the XXVIth and succeeding neuromeres are single, and the details of their fusion have already been described under the "Leydig's cell." The arrangement of the capsules is interesting, for they help to give evidence of the relations between Clepsine and Nephelis (Fig. 1 in the text). The ventral capsules of a normal neuromere lie one in front of the other, so that the line separating them is transverse. This obtains in XXV, XXVI, and XXVII, the first three neuromeres of the "anal ganglia." But in XXVIII and the succeeding neuromeres the ventral capsules lie side by side, the line separating them being longitudinal.

[15] Ph. François: Contribution à l'étude du système nerveux central des hirudinées. Poitiers. 1885.

In discussing the anal region of Clepsine, in which the same condition obtains, Whitman says (9, p. 388): "This arrangement, evidently one of mechanical adjustment necessitated by the shortening and crowding of the segments, prevails throughout the caudal region with the exception of the first segment (XXVIII) in which the sacs are placed one behind the other as in typical trunk segments."

Again, in his description of *Clepsine plana* (16, p. 413), he says: "Reduction, as I have before pointed out, seems to have begun at both extremities, and to have advanced from these points towards the middle of the body. Its advance shows how far a form has departed from the ancestral condition of uniform somites. It is here that we discover a very important guide to the systematic rank and relationship of different forms." These seven neuromeres, then, correspond to the entire anal ganglia of Clepsine, as is shown by the degree of fusion in the lateral nerves and in the arrangement of the ventral capsules. The process of reduction in the anal region has gone on further in Nephelis by three metameres than in Clepsine, while in the head region the number remains the same in both forms.

The Head Region.

In order to make an analysis of the terminal somites of the head region we must keep in mind that the external criteria of a neuromere are six capsules, two being ventral, two pairs of nerves, and a pair of "Leydig's cells." Beginning at the posterior end and working forwards we shall have little, if any, difficulty in finding six neuromeres.

The nerves of the last neuromeres, VI (Pl. VI, Figs. 10–13), arise as single trunks, but divide very near the body of the ganglion, and in the angle of separation of each trunk lies a "Leydig's cell." Two pairs of lateral capsules, 6.6., separated from the others, are easily identified as belonging to this nerve, so that with the two end capsules of the two ventral series we find all the elements of the typical body neuromeres. The next nerve, V, arises as a single trunk and proceeds forwards as

[16] "Description of Clepsine plana." *Journ. of Morph.*, vol. iv, 1891.

such until it passes the collar, when it divides into a ventral and dorsal branch, and at this point of separation, as in VI, lies a "Leydig's cell" (Pl. VII, Fig. 16). Two pairs of lateral capsules lie well separated from the others, just anterior to those belonging to VI, and two more, 5.5., of the ventral series furnish the elements of this neuromere, V. The next nerve, IV, arises as a single trunk, proceeds for a much longer distance as a single trunk, sending off to the 5th annulus a dorsal branch which quickly divides (Pl. V). This annulus is a very narrow ring lying in the plane of flexion of the oral sucker and the body. The "Leydig's cell" of this neuromere lies completely outside of the nerve trunk, just as it does in XXVIII or the 4th anal neuromere, and sends one fiber forward and one backward (Pl. VII, Fig. 16). The two pairs of lateral capsules belonging to the neuromere lie just anterior to those of neuromere V close to the angle made by the collar. The third capsule of the cluster at this point, lying close to, and anterior to, these two, belongs to the next neuromere, III (Pl. VI, Figs. 11 and 13). These two lateral pairs of capsules, together with two, 4.4., of the ventral capsules, complete the elements of neuromere IV. The next nerve, III, arises just anterior to IV and proceeds in much the same manner, dividing near annulus 5 into dorsal and ventral branches. The "Leydig's cell" lies alongside the trunk, as in IV (Pl. VII, Fig. 16). The lateral capsules belonging to this neuromere show the same peculiarity that Whitman found in Clepsine and that I have seen in Macrobdella,— one pair lying close to the capsules belonging to neuromere IV, while the other pair lies close to the capsules of II, being separated by a wide space. The two ventral capsules, 3.3., complete the elements of this neuromere (Pl. VI, Figs. 11 and 13).

The next nerve trunk arises from the collar as a single large trunk and proceeds some little distance before it shows evidence of separation, and just after separating a "Leydig's cell" appears on each trunk as in III and IV (Pl. VII, Fig. 16). We have here, then, nerves II and I as their distribution also shows. The lateral capsules of II are situated on the posterior side of the collar, while the most anterior, 2.2., of the ventral capsules

complete the elements of this neuromere. The capsular elements of neuromere I differ from all the others, in that the whole six are carried on the dorsal part of the collar (Pl. VI, Figs. 11 and 13). Excepting the position of the ventral capsules, the supra-oesophageal ganglion does not differ from the typical neuromere, and the argument made by Whitman (9) for Clepsine applies with equal force to Nephelis. Not only do these nerve trunks, "Leydig's cells" and capsules, show by their analysis the presence of six, and only six, neuromeres in the head region, but the distribution in the peripheral parts confirms it and sets the limits to the terminal somites in the most conclusive manner.

The gold chloride stain was peculiarly valuable in this work, and gave me sections with which it was only a question of patience to follow out the well-defined nerve branches to their peripheral parts. The fibers stand out distinct in form and color, not to be confused with any other element in the head. The spherical cysts of a parasitic nematode often furnished excellent data for the perfect superposition of the drawings of a series of sections and made it possible to follow out every fiber represented in my drawings through its subdivisions to the sense organs.

Beginning, as before, at the 11th annulus (Pl. V, Fig. 2) I find the distribution from behind forward as follows: the 11th annulus contains an intermuscular nerve ring, and receives its innervation from the succeeding neuromere, VII. The posterior trunk nerve of VI sends a ventral branch to the 10th annulus and a dorsal branch which innervates dorsal sensillae on the 10th and 9th annuli, as well as sending a branch forward to the intermuscular nerve ring of annulus 8. The anterior branch is wholly ventral and lateral, innervating the intermuscular nerve ring in the 8th annulus and a few ventral sensillae. The 9th, 10th, and 11th annuli, therefore, make up metamere VI, the innervation of which is strictly comparable to that of a body metamere, being dimeric and triannulate. The most striking departure from the five-ring metamere lies in the absence of the intermuscular nerve ring from annulus 10, morphologically the 2d annulus of the

body metamere. Proceeding forwards, the 8th annulus has an intermuscular nerve ring, innervated as has just been described from the succeeding metamere, VI.

The inner, or median, branch of nerve V corresponds to the anterior lateral nerve of a body somite and innervates a few ventral sensillae on the 7th annulus, the outer lateral sensillae of annulus 6, the ventral portion of the intermuscular nerve ring in annulus 5, and thence passing forwards innervates the labial sense organs on the ventral margin of the oral sucker. The outer branch, corresponding to the posterior lateral nerve, rises sharply to the dorsal side (Pl. VII, Fig. 16), innervates the sensillae in the 7th and 6th annuli, and sends a branch to the intermuscular nerve ring in the 5th annulus. The 6th, 7th, and 8th annuli, then, form metamere V, and again we find the dimeric and triannulate distribution found in Clepsine and in the normal body metamere of Nephelis. From this metamere forward the distribution is simpler but readily referable to the body metamere. Annulus 5 is, as has been described, very narrow and situated in the plane of flexion, yet it represents metamere IV, for it contains an intermuscular nerve ring innervated from the succeeding somite, and nerve IV gives off a dorsal branch which, quickly dividing, innervates dorsal sensillae and the third pair of eyes in this annulus, while the ventral branch goes forward to innervate some of the lateral labial sense organs. The persistence of this annulus in the plane of flexion is a striking instance of the stability of the 1st annulus of the metamere. Reduced, by its position, to the narrowest annulus in the animal, so narrow that the eye belonging to it has been forced partly outside of it into the broad 4th annulus, it retains not only the characteristic features of the 1st annulus, but also the intermuscular nerve ring belonging to the posterior annulus of the normal body metamere. Annulus 4 is broad and bears two rows of large sensillae on its dorsal surface. It represents metamere III. Nerve III divides as it enters the annulus, sending off a dorsal branch, which soon divides, one branch going to the sensillae of the annulus, the other innervating the second pair of eyes. The ventral branch goes to labial organs on the dorso-lateral margin.

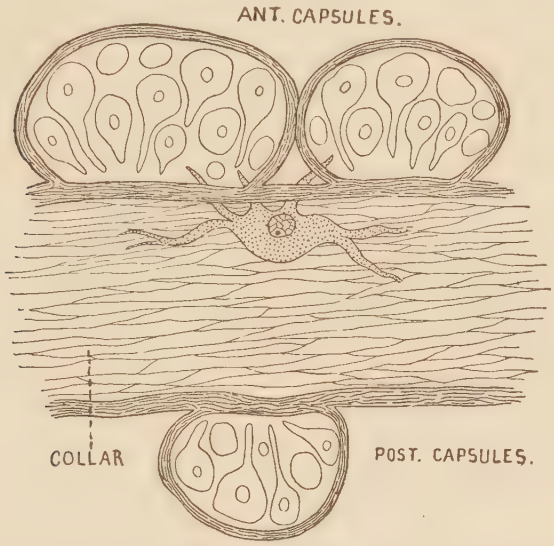
I have found evidences of the intermuscular nerve ring in this annulus, but I have not been able to trace them completely enough to describe the relations which the ring bears to the other parts of the nervous system.

The annuli lying in front of annulus 4 are incomplete, as shown in Pl. VI, Figs. 5 and 6. Annulus 3 is well marked off on the dorsal side, and the groove separating it from 2 is clear and sharp in outline, while 1 is separated from 2 by a partial groove extending about two-thirds of the way across the dorsal surface.

Nerve II innervates the numerous, large, dorsal sensillae of annulus 2, a few small ones on annulus 3, and the large first pair of eyes in annulus 2. The ventral branch of this nerve is reduced to a small branch that traverses the long axis of the first eye and proceeds to a few of the dorsal labial organs. Annuli 3 and 2, therefore, make up metamere II, and the nerves of this metamere, like those described, innervate the preceding metamere. Nerve I innervates annulus 1, supplying the numerous large sensillae and the numerous mid-dorsal labial organs. This fact raises the prostomium to the rank of a metamere, and it must be counted as one. It has been customary to disregard this reduced portion in numbering the metameres and annuli, but hereafter it must be reckoned in the count of metameres and annuli, as Whitman has done in Clepsine (*l. c.*).

Thus far the external features of the sub-oesophageal ganglia and the "brain" and the distribution of the nerves have been analyzed with concordant results; there remains an internal factor that adds still further proof for Professor Whitman's proposition. In Nephelis, as in Clepsine and other Hirudinea, each body neuromere contains, as I have said, two "median nerve cells." In Nephelis, as in Clepsine, they are found in the sub-oesophageal portion, but arranged numerically, four pairs appearing instead of five, as Whitman finds in Clepsine. Careful examination of excellent sections reveals further that in each side of the collar, near the capsules, 2.2., ascribed to metamere II is a "median nerve cell" somewhat irregularly compressed. The volume of the cell is still large, and the

nucleus has the same size and characteristics of the typical "median nerve cell." Still further dorsally, lying between the capsules ascribed to metamere I, I find the pair of remaining "median nerve cells" here compressed into a spindle



TEXT-FIG. 2.—The "median" cell in neuromere I as seen in a horizontal section through the dorsal part of the collar. The section passes through the capsules, 1.1.1., of Fig. 13, Pl. VI. Camera outlines. $\frac{1}{12}$ immersion oc. 3. Reduced one-half.

form (Fig. 2 in the text). This discovery enables us now to say, without reserve, that every element recognized in the body neuromere is found in the supra-oesophageal ganglia, and, therefore, that the supra-oesophageal ganglion or "brain" is homologous with a body neuromere.

THE INTERMUSCULAR NERVE RING.

Intimately connected with the central nervous system and probably closely related to it in origin is a remarkable peripheral system of nerves hitherto, so far as I have been able to learn, wholly unknown and unsuspected by all investigators who have worked especially on the details of the nervous system of leeches. For the discovery, I must again thank the gold-chloride method of staining, for the only elements of it

that show in control preparations are the large bipolar cells, and these are constant whatever method is employed.

Traces of this system are found in the most anterior portions of the head in the form of large bipolar cells, whose connections I have not yet determined. The 1st ring occurs in metamere IV, annulus 5; the 2d in metamere V, annulus 8; the 3d in metamere VI, annulus 11, the last annulus of the head region. From this point onward two rings are found in each full metamere, in the 2d and 5th annuli respectively. I have not found the ring behind metamere XXIV, or any well-defined traces of it.

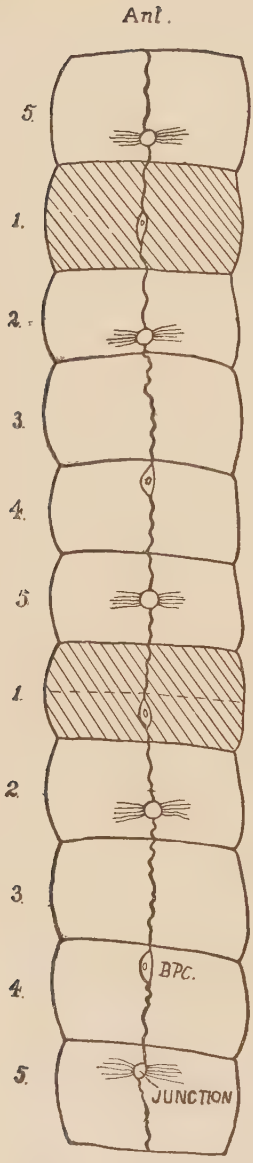
Beginning with metamere VII, I find eight bipolar cells connecting each ring with the succeeding one, as shown in Pl. V and as will be described below. Each ring receives fibers from, and sends fibers to, the central nervous system, and fibers from the ring run to sensillae and muscles.

Figure 18, Pl. VII, is a diagram of one-half of a transection made in the mid-body region, designed to show the arrangement and relations of this nerve ring. It is a projection constructed from camera drawings of the nervous elements in annuli 5 and 1 of adjoining somites upon a single plane, viewed from anterior to posterior. The anterior nerve (compare Pl. V, Fig. 2) runs laterally in the 1st annulus, sending forward two ventral branches, between the 3d and 4th, and the 4th and 5th bundles of long muscles, one to the nerve ring in the 5th annulus, the other, the cut end of which is shown in this figure, to a few ventral sensillae in the 4th annulus. Passing out to the edge, it innervates three large sensillae in annulus 1, as shown. The posterior nerve rises dorsally and innervates three large sensillae on the dorsal surface of annulus 1, and sends forward a branch which goes to the nerve ring between the 4th and 5th bundles of dorsal long muscles and on to a few dorsal sensillae in the 4th annulus. The nerve ring here represented lies in the 5th annulus. It consists of a complete ring of fibers which lie between the layers of long muscles and the circular-oblique muscle layers, whence the name I have given to it. Around this ring at definite points are ten groups of bipolar cells, six

on the dorsal side, four on the ventral side. These cells resemble in size and character of nucleus the "Leydig's cell" of the body neuromere. They all send fibers in both directions into the ring, but I have been so far unable to follow them to their terminations. The groups differ in characteristic features, and for the purpose of description I have named them as shown in the figure. They are constant in their position and character throughout the leech, and this I determined by comparing the six rings in three successive mid-body metameres minutely, detail with detail, by the method described in the early part of this paper.

The inner dorsal group (*in. d. bpc.*) consists of one bipolar cell with a short pedicel, lying in the 3d muscle bundle almost sessile on the ring. The outer dorsal group (*o. d. bpc.*) consists of a cell with a very long pedicel, lying in the 4th muscle bundle. The lateral dorsal group (*lat. d. bpc.*) consists of six or eight cells, four to six of which are small, lying in the outer or 6th muscle bundle.

The outer ventral group (*o. v. bpc.*) consists of one cell with a short pedicel, lying in the 5th muscle bundle, and like the inner ventral group, very near the point of junction with the nerve trunk from the central system. The inner ventral group (*in. v. bpc.*) consists of two or three cells with pedicels of medium length, lying near the edge of the 4th muscle bundle. The connection with the central system at this point is different from the others. The trunk divides just before reaching the ring, and as I have traced fibers from this inner ventral group into that branch nearest the group, it is evident that it carries fibers from these cells to the ganglion, the sensory fibers, while the other branch of the nerve trunk carries motor fibers from the ganglion into the ring. If my data as just given are sufficient, then we have the same morphological conditions that obtain in the spinal ganglion of vertebrates. The one piece of evidence wanting is the termination of the other pole of the cell. These cells are without doubt those which Retzius (*l. c.*) sought, as quoted above (page 42), to find in the epidermis. These cells, together, possibly, with the outer ventral cell, answer the conditions called for by Retzius for



Text-fig. 3. — A very narrow tangential section showing the long bipolar cells running from junction to junction through two metameres and five rings. (Camera drawing from a gold chloride preparation.)

those central endings which enter by the anterior nerve. I have not been able to determine which of the dorsal cells send in fibers by way of the posterior nerve. The ring itself, as shown in the gold-chloride preparations, consists of numerous fine fibers, which come in part from the bipolar cells, in part from the central system, and in part from the diffuse sensillae in the epidermis. It gives off fibrillae which, ramifying the bundles of long muscles, innervate the cells.

As I have said above, these rings are connected, one with another, by long bipolar cells lying between the same muscle layers, as shown in Pl. V. These connecting cells form, when taken together, eight longitudinal paths, reaching, according to my present investigations, from metamere VII through the body region to metamere XXV. I have no doubt that they may be found in the regions of the terminal somites, but I have not yet been able to do so.

These long connective cells join the nerve rings at the points where the branches from the central system come into the ring (Fig. 3 in the text). Every point of connection of the ring with the central system is also a point of junction with two long bipolar cells. I regret exceedingly that the histological character of this junction is wholly obscured by the swelling of the tissues by the formic acid, and I hope to study this detail by the use of Golgi's method of silver impregnation or methylen

blue. The bodies of the cells lie about midway between the nerve rings (Pl. V, long *bpc.*), and present the same general appearance in size and nucleus as the bipolar and "Leydig's cells."

The physiological rôle played by this highly specialized peripheral system is, doubtless, of the utmost importance, and anything like a discussion of its functions can be made only after the details of its constitution have been more fully worked out, and something is known of the comparative anatomy of the structure in other worms. It plainly offers a method for short reflexes, such, for instance, as those controlling the rhythmic undulating motions of the leech when at rest, and supposed to be respiratory; or for the successive stimulation of the muscles in voluntary motions. The presence of this system may throw some new light on the phenomena that go under the name of "skin tension."

Leaving its physiological functions for another investigation, its presence may be brought to bear testimony on the question of the derivation of the five-ring form of metamere. In his *Metamerism of Clepsine,* Whitman says (p. 392): "In my description of *Clepsine plana* (1891) the following note may be found (p. 414): 'I am reminded of an error into which I fell in my paper on Japanese leeches. The error was the assumption that all somites having less than five rings were abbreviated. The assumption should have been, as I now feel convinced, that all somites with less than three rings are abbreviated, and all with more than three have been increased by the division of one or two of the three primary rings. I have collected considerable evidence, which cannot be given here, to show that in the evolution of Hirudo it was the *2d and 3d rings that underwent division,* while the 1st remained undivided.'" On page 393 he continues, under the head of "Multiplication of annuli": "It is a fact of some importance, in estimating the morphological value of the metamere, that the multiplication of annuli seems to follow the same general law as the multiplication of metameres in the embryo; that is to say, the *posterior end* of the metamere, like that of the embryonic trunk, is the region of most rapid growth and elon-

gation, and the new rings are added by the division of the ultimate (3d) ring alone, or by the division of both the ultimate and the penultimate, somewhat as new metameres are added by the division of the part lying behind the last one formed. There is not then a uniform growth throughout the trunk, but a curve for each metamere."

When I first found the intermuscular nerve ring I made a very careful search in the 3d and 4th annuli for every trace of nerves, and found that they were very weak in those structures, and when I came to plot down the nerve ring as it occurs in the successive metameres, it became evident that the 5th and 2d annuli of each somite (see Pl. V) were in strong and equal connection with the 1st annulus which carries the ganglion.

The absence of anything like a proportional division of nerves between the several annuli shows, I believe, that the annuli weak in nervous elements are the younger and secondary annuli, formed by the division of the 2d and 3d rings as follows: the posterior half of Clepsine 2 becomes 3 in Nephelis, and the anterior half of Clepsine 3 becomes 4 in Nephelis. This mode of formation of the five-ring type of somite does not involve any shifting of the nephridiopore, as would happen if the posterior half of primitive 3 became 5 in Nephelis.

This explanation assumes, of course, that the intermuscular nerve ring is a constant feature in the leeches, and I feel confident in predicting its discovery not only in the leeches, but either that or its homologue in other annelids as soon as they are studied with good nerve methods. There are many evidences in the structure of the ring that it is an old and very stable structure. The constancy through successive metameres of such features as a long pedicelled cell always in the same position on the ring ; the group of sensory fibers separated from motor fibers near the inner ventral group of bipolar cells ; the strong innervation from the central system, and the definite longitudinal connectives, all point strongly to the nerve ring, as we find it in Nephelis, as being very highly specialized and the resultant of two originally distinct systems.

In Lumbricus, for instance, both the afferent and efferent fibers of the central system, though somewhat more diffuse than in Nephelis, run in well-defined bundles, and Miss Langdon (17) has found numerous bipolar cells along these nerves. These two elements, fibers and cells, necessary to form the intermuscular nerve ring of Nephelis are present in Lumbricus, and when one takes into consideration the vast differences between the life habits of the sluggish, mainly herbivorous, earthworm and the active, free-swimming, carnivorous Nephelis, it does not seem difficult to believe that specialization, so far advanced among the leeches in other particulars, may so combine these factors as to produce the result found in Nephelis. This suggestion by no means excludes any other explanation; it is the one nearest at hand in the light of our present knowledge. Recent investigations, with methylen blue especially, show that the peripheral bipolar ganglion cells connected with the central system play an important part in the neural system of some of the flat worms, and the whole matter of peripheral nerve systems in this group, as well as that of the annelids, is now in such a promising condition of investigation that much light will, doubtless, soon be thrown upon it.

The "Large" Nerve Cells.

Investigators of annelid and arthropod nervous systems have been familiar for a long time with certain nerve cells so large in comparison with the ordinary motor cells of a ganglion that they have often designated them by such words as "giant," "colossal," and the like, and have described their location, character, etc., without, so far as I know, bringing them into any relation with each other. Such a relation exists in Nephelis, though what its full significance may be I do not yet know. I find the large cells in a body somite arranged as follows: in the ventral chain two "median" or "giant" cells *within* the ganglion; in each connective *between* the ganglia lies a "colossal" axial cell, which sends processes before and behind into

[17] Fanny E. Langdon: "The Sense Organs of Lumbricus agricola Hoffm." *Journ. of Morph.*, vol. xi, 1895.

the ganglion; therefore, *near* the "median" cells. In each intermuscular nerve ring are about twenty-two "large bipolar" cells, some of which send processes *into* the ganglion in the neighborhood of the "median" cells, while the eight "connective" bipolar cells joining the two rings terminate in some manner in close proximity to the fibers of the nerve ring at their junction with the ring. This leaves but one "large" cell yet to be accounted for, the "Leydig's cell" near the ganglion. This is a bipolar cell, and the processes may be readily distinguished as they pass outward on each lateral nerve *toward* the periphery. These processes, however, soon fuse with the nerve trunks, and I have not been able to follow them to any considerable distance. It is quite significant that these same trunks, or branches from them, send fibers into the intermuscular nerve ring, and furnish a path by which the processes of this cell may reach to the other large cells. If it does not come into proximity with the others, it forms an exception to all the other "large" cells in the somite.

Another fact to be noted of all these cells, numbering nearly fifty in each somite, is that the nuclei are practically the same in size and character, and the volume of the different cells is, so far as sections show, practically the same, whatever the shape may be. Such a definite arrangement cannot be without a purpose, the significance of which may in some measure be revealed by their development, and this I hope to determine soon.

In brief, if by some means we could remove all other cells and tissues in a somite excepting the "large" nerve cells and leave them in their normal relations, we should find them all joined together, forming a closed system capable, on the one hand, of receiving impressions and stimulating muscles independently, and, on the other hand, so related to the central nervous system through the cells in the ganglia and connectives as to make it completely adjunct to it.

THE SYMPATHETIC SYSTEM.

Leydig (18) and others have found evidences of a sympathetic system arising from the collar in certain leeches and other

[18] F. Leydig: Tafeln zur vergleichenden Anatomie. Tübingen. 1864.

annelids. In Nephelis, I have found it to be much more extensive than has been figured in any annelid that has come to my notice.

It arises in Nephelis very similar to the method figured by Leydig (*l. c.*) for *Haemopis vorax* Brandt (Pl. IV, Fig. 5). In this latter leech Leydig shows the sympathetic system lying on the walls of the "crop," but not connected with the part arising from the collar. In Nephelis (Pl. VII, Fig. 17) the junction between the two systems is on the median side of the collar near the nerve root I–II. A fibrous projection from the anterior side of the collar on each side gives rise to three branches which run over the wall of the oesophagus; the dorsal and ventral roots pass off in a ⊣ fashion, while the lateral root comes off from the median side. Six capsules, three on either side, contain nerve cells whose processes run into these branches. One pair (Pl. VI, Figs. 11 and 13, symp.), the larger of these capsules, is on the collar, the other two on the posterior side of the dorsal branch. The ventral branches retain their individuality for some distance as they approach the mid-ventral line, but they soon become lost in a system of closed meshes. The lateral branches continue as such, plainly taking part in forming the meshes, but preserving their identity throughout (Pl. VII, Fig. 17; Pl. VIII, Fig. 19).

The dorsal branch of each side rises parallel to the collar, just in front of it. Professor Patten has called my attention to the fact that this structure in this position is comparable to a similar structure in Limulus. A narrower band connects them in the mid-dorsal region so that they form together a half circle. Two branches pass backward near the median plane into two large ganglionic masses lying just under the collar (Pl. VIII, Fig. 20). All these branches give off bundles of fibers that run forward to the buccal cavity, and these bundles differ in two ways from the plexuses behind the collar. They contain but few, if any, cell bodies, being processes of the cells that lie clustered together in ganglionic masses between the fibers and the meshwork of the plexuses on the oesophageal walls. Fig. 20 shows this as it occurs on the dorsal side. It is a dorsal view drawn from a Haller preparation and, hence, shows no

cells. The same characteristic difference between the fiber bundles which run forward and the plexuses is found at each of the other branches, and similar but smaller ganglionic masses are present. Back of the collar, the muscular wall of the alimentary canal is covered with a complicated meshwork, as shown in Pl. VII, Fig. 17, and shown in greater detail in Pl. VIII, Fig. 20. The cells are multipolar and send processes in various directions, forming meshes. The processes ramify the wall and innervate the muscle cells. The preparations made with formic acid are not satisfactory for histological detail, and Fig. 20 is introduced to show the distribution, not the structure.

This system continues over the whole alimentary tract in substantially the same manner as shown on the oesophagus, and though from theoretical considerations I expected and sought diligently for metameric connections from the central system, I am confident that none exist.

In a few very favorable sections I have seen what I believe are traces of the sympathetic system in the post-anal region, extending in the axial line of the acetabulum and the sucker. The musculature in this region is so complicated that I cannot determine this point to my complete satisfaction. Nerve cells and fibers are certainly there and show plainly. There is no theoretical objection to their being a part of the sympathetic, for if in the ancestral form the anus was terminal and the sympathetic system was present to the anus, then in the leech the formation of the sucker undoubtedly made demands upon the muscles of the alimentary tract that may have continued after the anus moved forward and the sucker became imperforate. Again, while feeding, the leeches always hold themselves fast by the sucker, and the stronger stimuli to the muscles excited by food in the alimentary tract, during a meal, may by this same system be communicated to part of the muscles of the sucker and may help to make the adhesion more effective.

Summary.

1. Nephelis differs from nearly all other leeches in the external topography of the somite. The prominent sense organs present in most genera are not easily visible in Nephelis, excepting a few somites in the anal region.

2. Color and color markings do not afford criteria for the determination of specific characters.

3. The body somites consist of five annuli as determined by the nephridiopores. The somites of the terminal regions, containing less than five annuli were determined by the innervation.

4. But one species of Nephelis came under my observation though the collections were made over a wide area of country.

5. The food supply is the controlling factor in the choice of location in a pond or brook.

6. The head region contains six somites; the body region, eighteen; the anal, ten, making thirty-four in all.

7. The anterior nerve of a body neuromere arises from two roots which fuse quickly; the anterior nerve is, therefore, the morphological equivalent of the first and second nerves of Clepsine, while the posterior nerve in Nephelis is the equivalent of the posterior nerve in Clepsine.

8. "Leydig's cell" is present in every neuromere from one to thirty-four.

9. The innervation of a body metamere in Nephelis is morphologically identical with that of Clepsine.

10. The three anterior neuromeres of the anal ganglia show evidences that reduction in Nephelis has progressed by so much more than Clepsine, whose anal ganglia are represented by the succeeding seven neuromeres. In the head region the number of neuromeres is the same in both.

11. The distribution of the nerves in the somites of the terminal regions is precisely referable to that of a body somite.

12. In both terminal regions each neuromere contains every element found in a body neuromere.

13. A peripheral system of nerves composed of large bipolar cells, which I have called intermuscular nerve rings, is in intimate connection with the central system.

14. Some of these cells supply the fibers which Retzius and Biedermann describe as ending in the ganglion.

15. These rings are connected longitudinally by other bipolar cells which thus form direct axial paths for nervous impulses in addition to those furnished by the central system.

16. The relation of these rings to the distribution of nerves in a body metamere affords striking proof in favor of Whitman's theory of the formation of a metamere with five annuli from one of three annuli, that is, the posterior half of annulus 2 in Clepsine becomes annulus 3 in Nephelis; the anterior half of annulus 3 in Clepsine becomes annulus 4 in Nephelis, while the posterior half becomes annulus 5.

17. There is some evidence that this peripheral system of nerves is an old and very stable structure, and will be found in the other leeches. There is already some evidence that it or its forerunner is present in other annelids.

18. The "giant" nerve cells in the two systems, central and peripheral, are in close relation to each other, and are strikingly alike in cytological characters.

19. The sympathetic nervous system is well developed, and is connected with the central nervous system at the "collar."

20. The branches at this connection form a nerve circle in front of the "collar," such as is found in the arthropoda.

21. The nerve cells in the sympathetic system are multipolar, the processes forming meshes over the wall of the alimentary tract.

22. There is some evidence that the sympathetic system persists in the post-anal region, extending in the axial line to the muscles of the concave side of the sucker.

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DESCRIPTION OF PLATE IV.

Reproduced through the kindness of Professor Whitman from the "Festschrift zum siebenzigsten Geburtstage Rudolph Leuckart's," Leipzig, 1892, being Pl. XXXIX of that volume and appended to Professor Whitman's article, "The Metamerism of Clepsine."

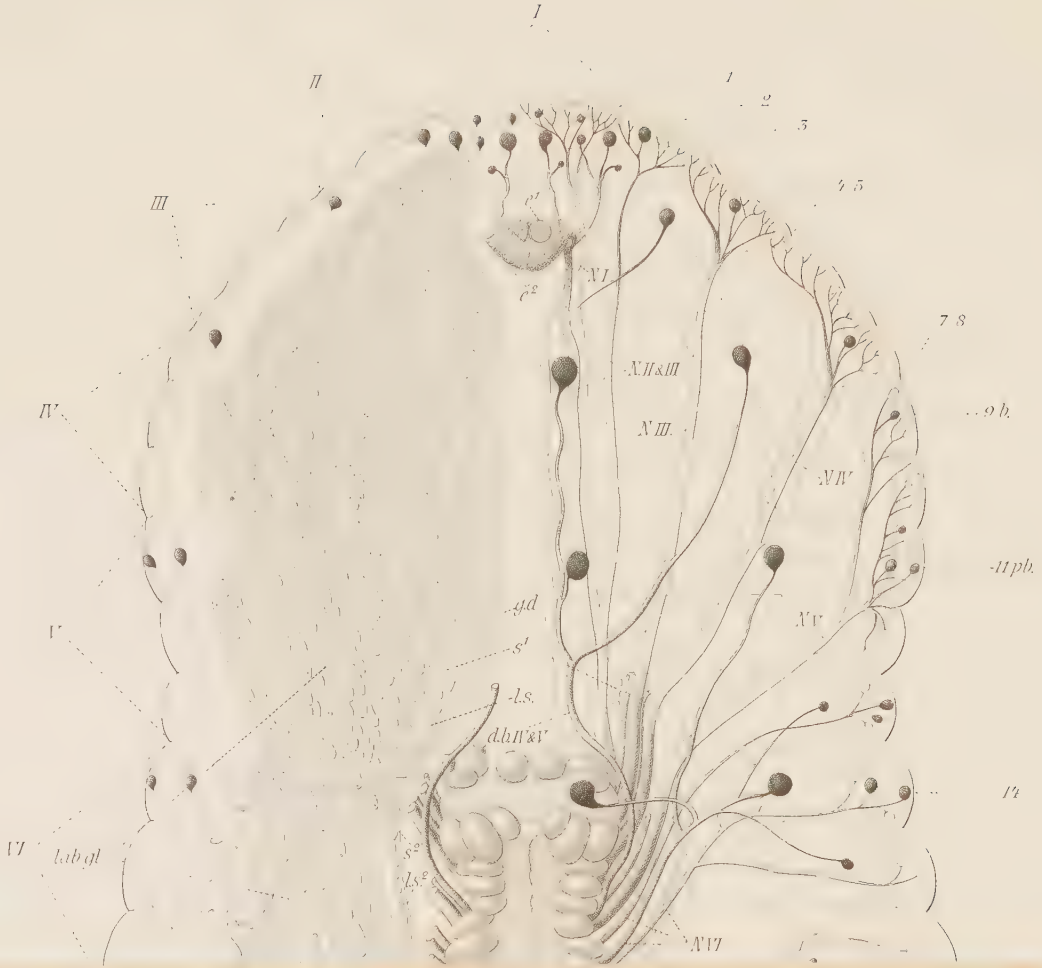
Representing the first eight segments as reconstructed from sections and surface views. × 50. The segments and nerves are numbered with Roman characters, the annuli with Arabic numerals. The metameric and smaller accessory sensillae of the dorsal side are represented in black, on the ventral side by circles.

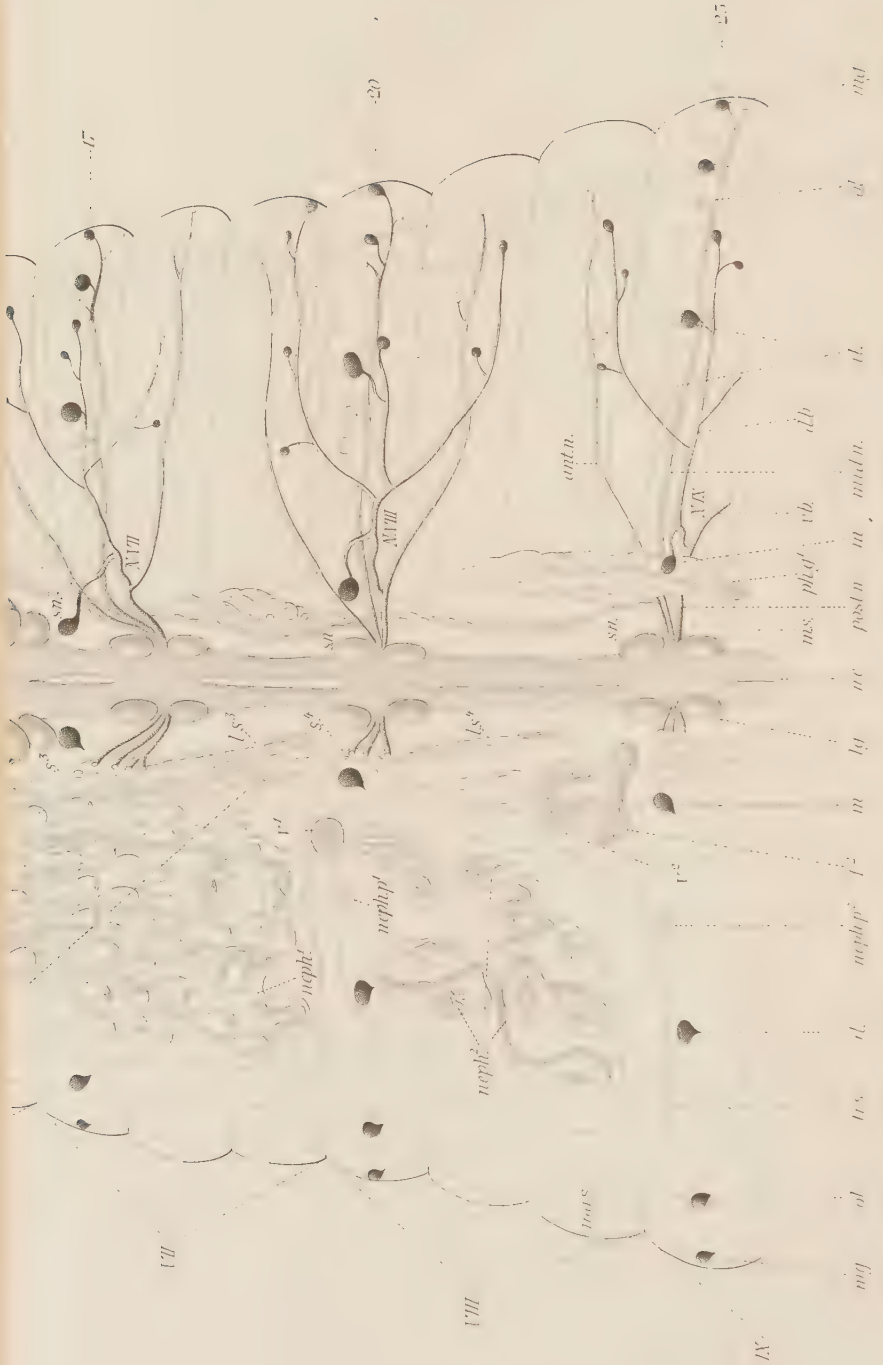
REFERENCE LETTERS.

Alphabetically arranged.

ant.n., anterior nerve. — *b.*, buccal annulus. — *d.b.*, dorsal branch of post. nerve. — *d.b. IV a. V*, dorsal branch of nerves IV a. V. — E^1, rudimentary eyes. — E^2, principal pair of eyes. — *f. 1* and *2*, nephridial funnels. — *g.d.*, bundles of gland ducts. — *i.l.*, inner lateral sensillae. — *lab.gl.*, labial glands. — *l.s.*, longitudinal septa. — *m.*, median sensillae. — *mg.*, marginal sensillae. — *mg.s.*, marginal sinus. — *m.s.*, median sinus. — *mid.n.*, middle nerve. — *n.c.*, nerve cord. — *neph. 1–2*, 1st and 2d nephridia. — *neph.p.*, nephridial pores. — *o.l.*, outer lateral sensillae. — *p.b.*, post-buccal annuli. — *ph.gl.*, pharyngeal glands. — *post.n.*, posterior nerve. — *s. 1–4*, septa. — *s.n.*, septal nerve branch. — *tr.s.*, transverse sinus. — *V 1–2*, funnel vesicles. — *v.b.*, ventral branch of posterior nerve.

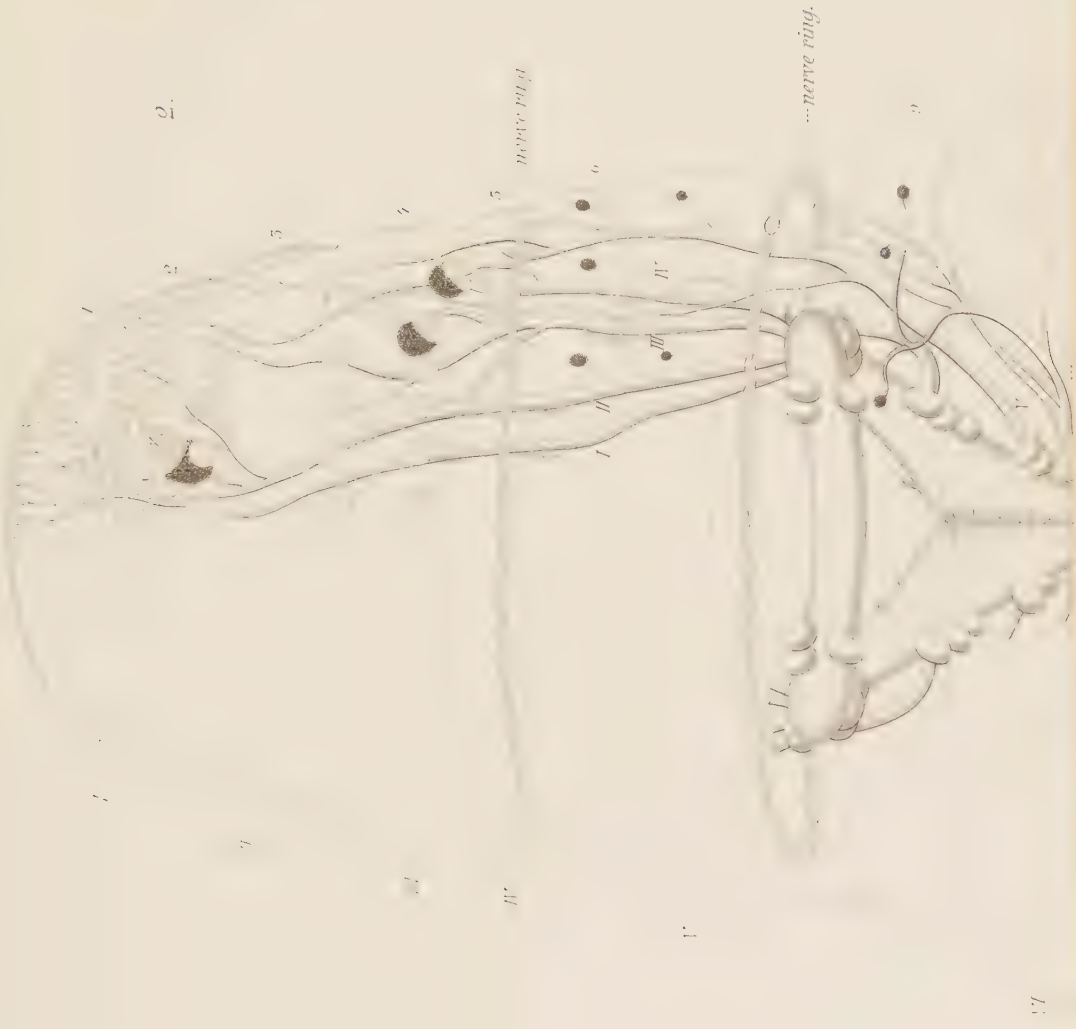
I.


I
II
III
IV
V
VI
1
2
3
4 5
7 8
c¹
c²
XI
XII&III
XIII.
XIV
XV
XVI
XVII
9 h.
11 ph.
12
gd
s¹
l.s.
d.h.IV&V
s²
l.s²
lab.gl

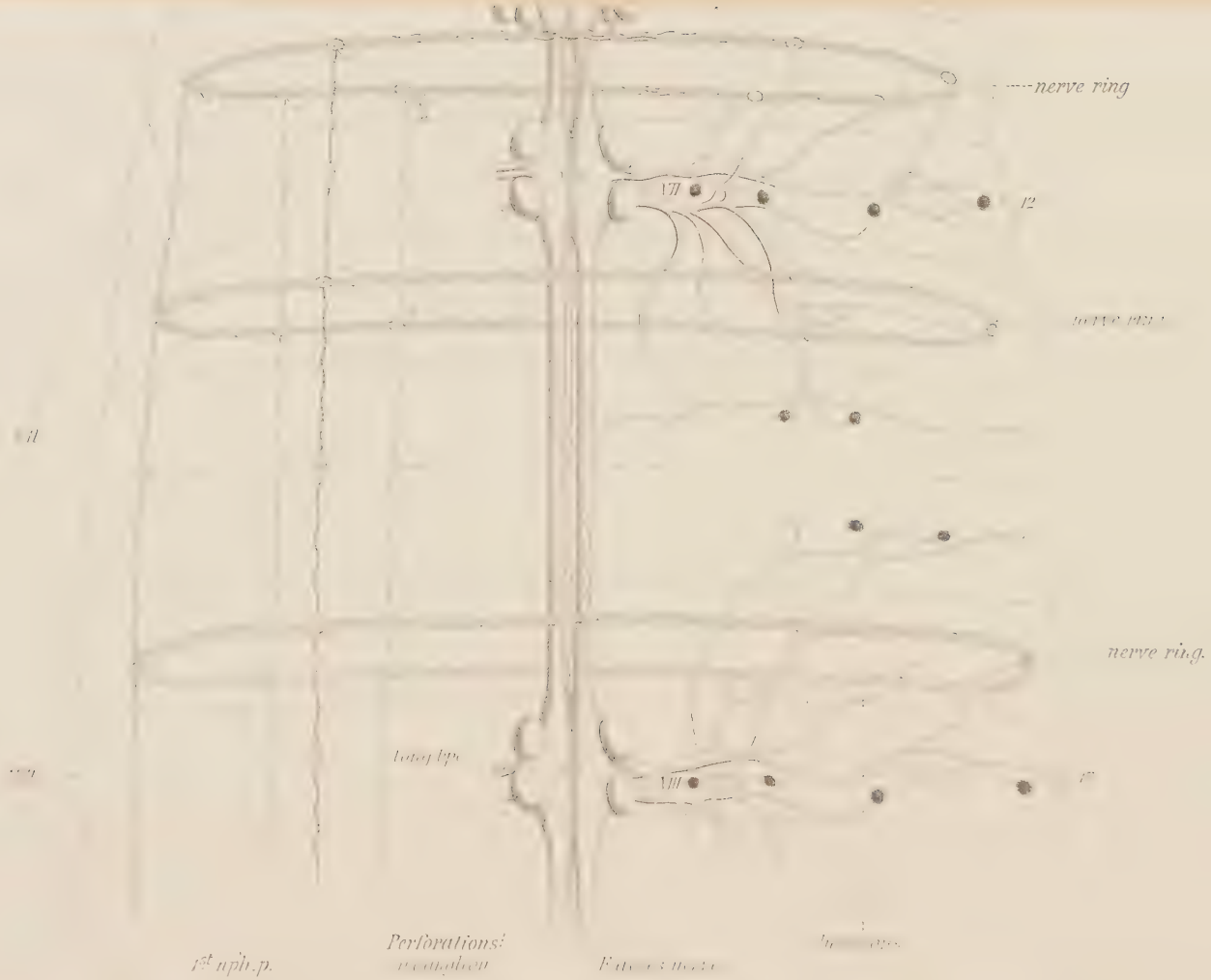

XVII
XVIII
XIX
sm.
sm.
sm.
nephr¹
nephr²
nephr¹
nephr²
nephr¹
nephr²
ves.
ves.
ves.

DESCRIPTION OF PLATE V.

Dorsal view of the central nervous system of Nephelis in the first seven and part of the eighth metameres as reconstructed from surface views and sections. × 30 (circa). The metameres are indicated on the left of the drawing by Roman numerals, the annuli are numbered by Arabic figures on the right. The nerves are also numbered with Roman numerals. Dorsal sensillae appear as black circles; ventral sensillae as light circles, excepting in the first five annuli, where they are drawn as they appear in a surface view of a specimen killed and examined in Haller's fluid. The details of distribution are shown so plainly as to do away with the need of explanatory references. On the left side, omitted from the right for clearness, are seen the long bipolar cells (long *b.p.c.*) that connect the intermuscular nerve rings. They are found at the junctions (Junctions) of the nerve branches with the rings, and the cut end of these branches are represented on that side. Faivre's nerve is indicated at *F.N.* The first pair of nephridiopores (*1st nph.p.*) lie between the 16th and 17th annuli.



C. E. Bricol, lith.


nerve ring
VII
12
nerve ring
nerve ring.
VIII
Perforations
1st uph.p.

Pl. V.

DESCRIPTION OF PLATE VI.

FIG. 3 is a diagram of *Nephelis lateralis* showing the boundaries of the somites in Roman numerals, the annuli in Arabic figures, the nephridiopores (*nph.p.*), the boundaries of the clitellum (*cl.*), and the sexual openings (male ♂, female ♀). The "brain" and the first two body neuromeres are sketched in to show their relative positions.

FIG. 4 is a dorsal view of *N. lateralis* from Wolf Lake near Chicago, Ill., showing the relative size of the annuli, and the number of sensillae as seen in a specimen freshly killed in weak chromic acid. The eyes are represented by crescentic black areas, as seen in a specimen killed in Haller's fluid. They represent the pigmented part only, the large, clear visual cells extending in a cone-shaped cluster from the concave side. The first pair look forward and outward, the second and third pairs backward and outward. Camera drawing: Zeiss, comp. oc. 1, obj. AA.

FIG. 5. Ventral view of the same showing the oral sucker as it appears in a freshly killed and well-extended specimen. Annulus 3 is the first complete annulus.

FIG. 6. Lateral views of the same showing the incomplete separation of annuli 1 and 2 and the doubling often seen in annuli 4 and 6.

FIG. 7. Anal region, dorsal view. Annuli 97, 102, 104, and 106 show well-marked sensillae in specimens freshly killed in weak chromic acid, though the number is not constant. Annulus 104 is double, the posterior half bearing the sensillae. The semicircular area on top of the sucker, the "acetabulum," is finely wrinkled, all traces of annulation being lost.

FIG. 8. Lateral views of an unusual specimen showing the partial fusion of the narrow annulus 5 with the broad annulus 4. Wolf Lake.

FIG. 9. Dorsal view of a normal body neuromere, showing the elliptical fibrous part in the middle, the two perforations in it near the median line, the slight groove in the median line passing between them. Faivre's nerve is shown as a narrow line between the two connectives. Two pairs of lateral capsules containing nerve cells lie on either side of the fibrous portion, one lying anterior to each of the lateral nerves. Two ventral capsules are shown under the fibrous portion. The dorsal and ventral roots of the anterior lateral nerve are shown together with the "Leydig's cell" lying between the two lateral nerves. (Nitric acid maceration; slightly stained with borax carmine.) Camera: Zeiss, obj. A, oc. 3.

FIG. 10. Dorsal view of the "brain," the sub-oesophageal ganglia, and first body neuromere, VII. (Nitric acid maceration.) Camera: Zeiss, obj. AA, oc. 2.

FIG. 11. Tracing of same. The capsules are numbered to correspond with the neuromeres to which they belong. The capsule designated "Symph" contributes its fibers to the sympathetic system only. It is not metameric.

FIG. 12. Side view of "brain" of same specimen, with same magnification.

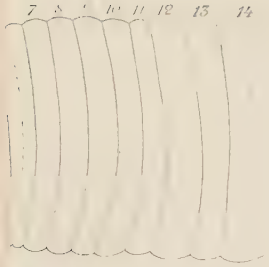
FIG. 13. Tracing of same; capsules numbered as before. This view shows the relations of the lateral capsules to the roots of their respective nerves in the "brain," and the same relations as they exist in the body neuromere, VII. The separation of the lateral capsules, 3. 3., of neuromere III is also shown. The position of the junction between the "brain" and the sympathetic is indicated on

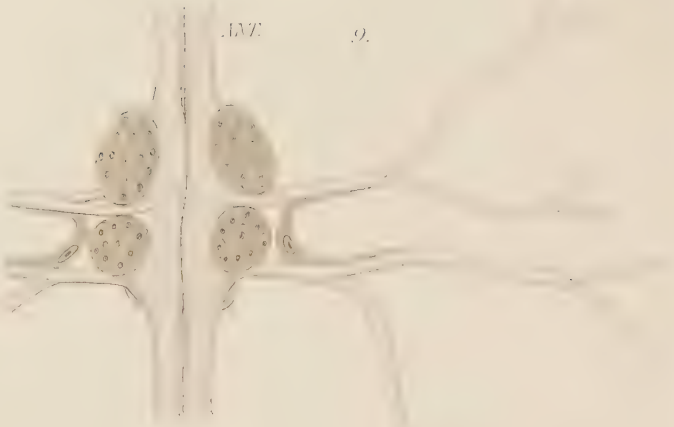
the median side of the collar at the level of the root of the nerves I, II. One large and two small capsules are connected with the sympathetic at each junction, which sends a dorsal and a ventral nerve bundle, shown here, and a lateral bundle not shown here, to the muscular wall of the alimentary canal. See Pl. VI, Fig. 17.

FIG. 14. "Anal ganglia," dorsal view.

FIG. 15. Ventral view of same. Metameres in Roman numerals. Capsules in Arabic figures. In Fig. 14 the crowding of the lateral capsules to the dorsal side begins with neuromere XXVIII, while the ventral capsules, Fig. 15, of the same neuromere are also crowded out of the normal. The seven neuromeres, XXVIII to XXXIV, constitute the entire "anal ganglia" of Clepsine. XXV is nearly normal, lateral nerves not fused; XXVI is slightly compressed, lateral nerves fused, but the two roots are plainly visible; XXVII is still more compressed. These three neuromeres indicate the extent to which abbreviation has proceeded farther in Nephelis than in Clepsine in this region.

3.
4.
5.
6.
8.
7.
brain
1st m.
1st sph.p
XV to XXIII omitted
17th sph.?
XXVII
XXVIII.
XXIX in
sucker.
sqm.ph





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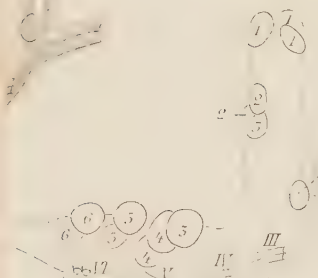

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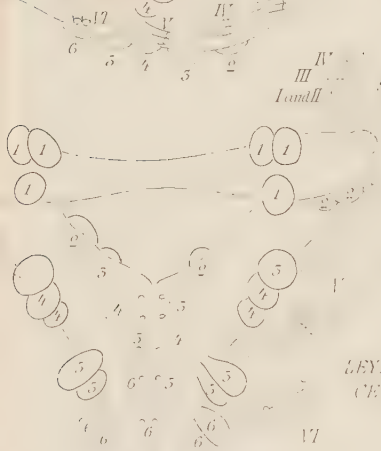
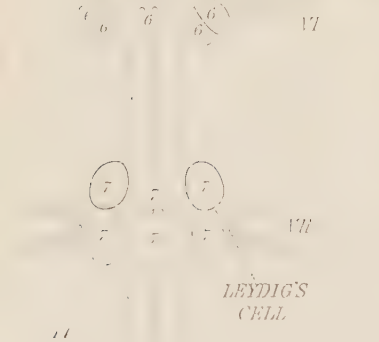
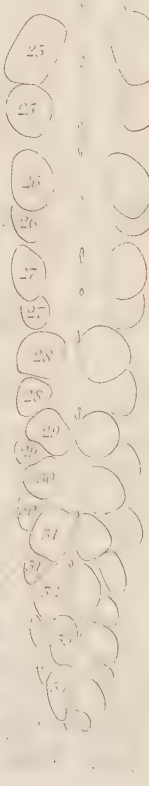
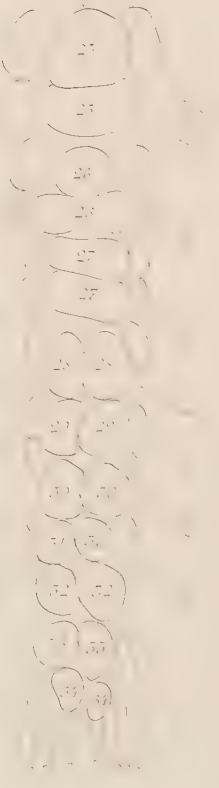


15







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DESCRIPTION OF PLATE VII.

FIG. 16. Lateral view of the nervous system of the head region, reconstructed from sections, showing the distribution and general paths of the principal nerve trunks, the intermuscular nerve rings in annuli 5, 8, and 11, together with their junctions with the central system, and other details. The principal eyes lie in annulus 2, the small ventral branch of nerve II passing through it axially to the labial sense organs. The second and third eyes look to the rear. On nerves I, II, III, and IV the "Leydig's cells" of those neuromeres lie outside of the nerve trunks which are the fused anterior and posterior lateral nerves of their respective neuromeres. In nerves V and VI the same cell is seen near the angle of separation of the fused parts.

FIG. 17. A reconstruction showing the general arrangement of the sympathetic system. Combined from sections and specimens killed in Haller's fluid. The body outline is drawn from a median section, the same as used in Fig. 16, and the parts of the central nervous system are traced in outline from Fig. 16. The right half of the oesophagus is shown covered with the closed meshes of the plexuses formed by the fibers from the multipolar cells. The cells are inadequately shown; see Fig. 20, Pl. VIII. Standing out from the median side of each half collar, median to the fused roots of nerves I, II, is a fibrous projection which gives rise to three trunks: (1) one going ventral and median, meeting the similar trunk of the other side in the mid-ventral line; they form anastamoses and gradually lose their outlines in the general plexus; (2) a lateral trunk that persists to the rear as shown; (3) a dorsal branch which runs parallel with the collar to the median dorsal line; the details of this arrangement are shown in Fig. 20. The dorsal trunk of each side carries two small capsules containing nerve cells, while a third capsule, whose fibers go into the sympathetic system, is located on the collar, not far from the origin of the connection between the central system and the sympathetic. Anterior to the collar the sympathetic system runs to the buccal cavity in fibrous bundles, apparently free from cell bodies.

16.

nerve rings.

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DESCRIPTION OF PLATE VIII.

FIG. 18. A projection of the nerves of the fifth and following first annuli showing especially the intermuscular nerve ring and its relations to the central nervous system. On the ring are ten (five in the half section) groups of bipolar cells designated as below. The trunks from the central system join the ring at the points marked 1 to 4 on the margin, which points also mark the points of junctions of the long bipolar cells with the ring. The numerals in the figure designate the bundles of longitudinal muscles, here seen in section, and drawn only in three of the ventral bundles. In these are shown fibrillae which leave the ring to innervate the muscle cells. The layers of circular and oblique muscles between the epidermis and the long muscles are omitted for clearness, in order to show the fibers from the sensillae to the ring.

ABBREVIATIONS.

al.	intestine.
br. to 1st ann. et seq.	branches of nerves to the respective annuli.
epi.	epidermis.
gn.	ganglion.
l.v.	lateral blood vessel.
med.bdl.l.m.	median bundle of long muscles.
m.circ. and ob.	layer of circular and oblique muscles.
sens. I, II, III.	three types of sensillae.
in.v.b.p.c.	inner ventral group of bipolar cells.
o.v.b.p.c.	outer " " " "
in.d.b.p.c.	inner dorsal " " "
o.d.b.p.c.	outer " " " "
lat.d.b.p.c.	lateral " " " "
1–4, marginal.	junctions of ring with central system and the long connective bipolar cells. See Pl. II, Junctions.
1–6, within.	ends of the bundles of long muscles.

Outlines taken from camera drawings.

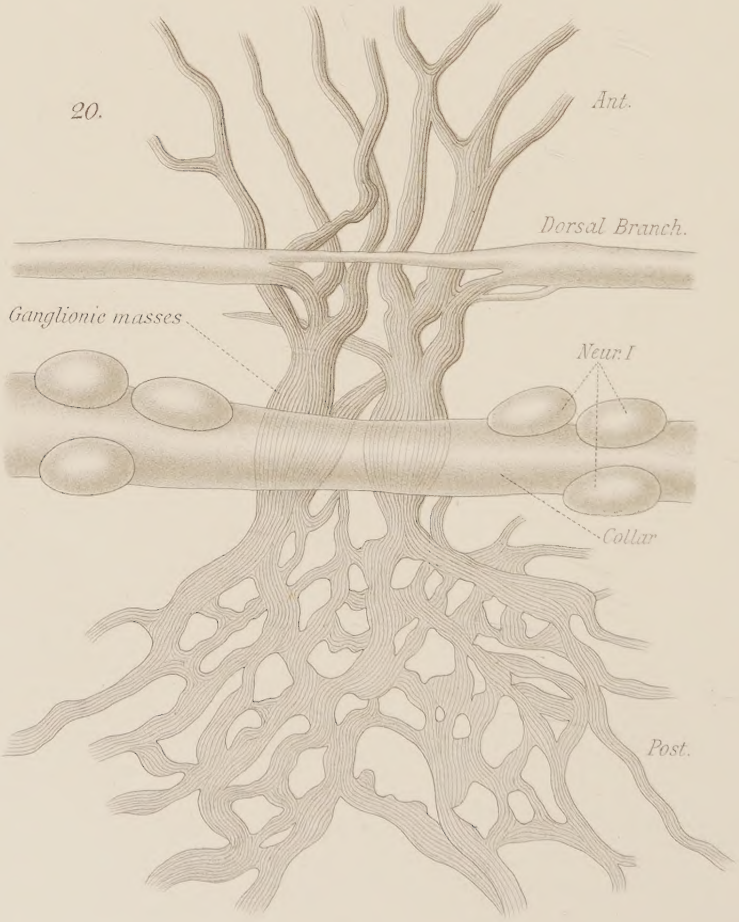
FIG. 19. Camera drawings of a section showing details of the sympathetic system. The faint circles are the cut ends of muscle cells. In many of them may be seen the nerve end plates. The wide bundle of fibers is part of the lateral bundle. See Pl. VII, Fig. 17. Reichert, $\frac{1}{12}$ immersion, oc. 3.

FIG. 20. Dorsal view of part of the collar and sympathetic system. From a flattened head killed in Perenyi's fluid and viewed as a transparent object in Haller's fluid. It shows the continuity of the dorsal branches of the sympathetic, the ganglionic masses under the collar (Pl. VII, Fig. 17), and the different character of the fibrous bundles running to the buccal cavity and the plexuses posterior to the collar. Zeiss, c. oc. 2, camera drawing, reduced nearly one-half.

C.L. Bristol del.

19.

20.



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